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Uncovering Mechanisms for Amyloid Precursor Protein Processing and Trafficking

Saket Milind Nigam

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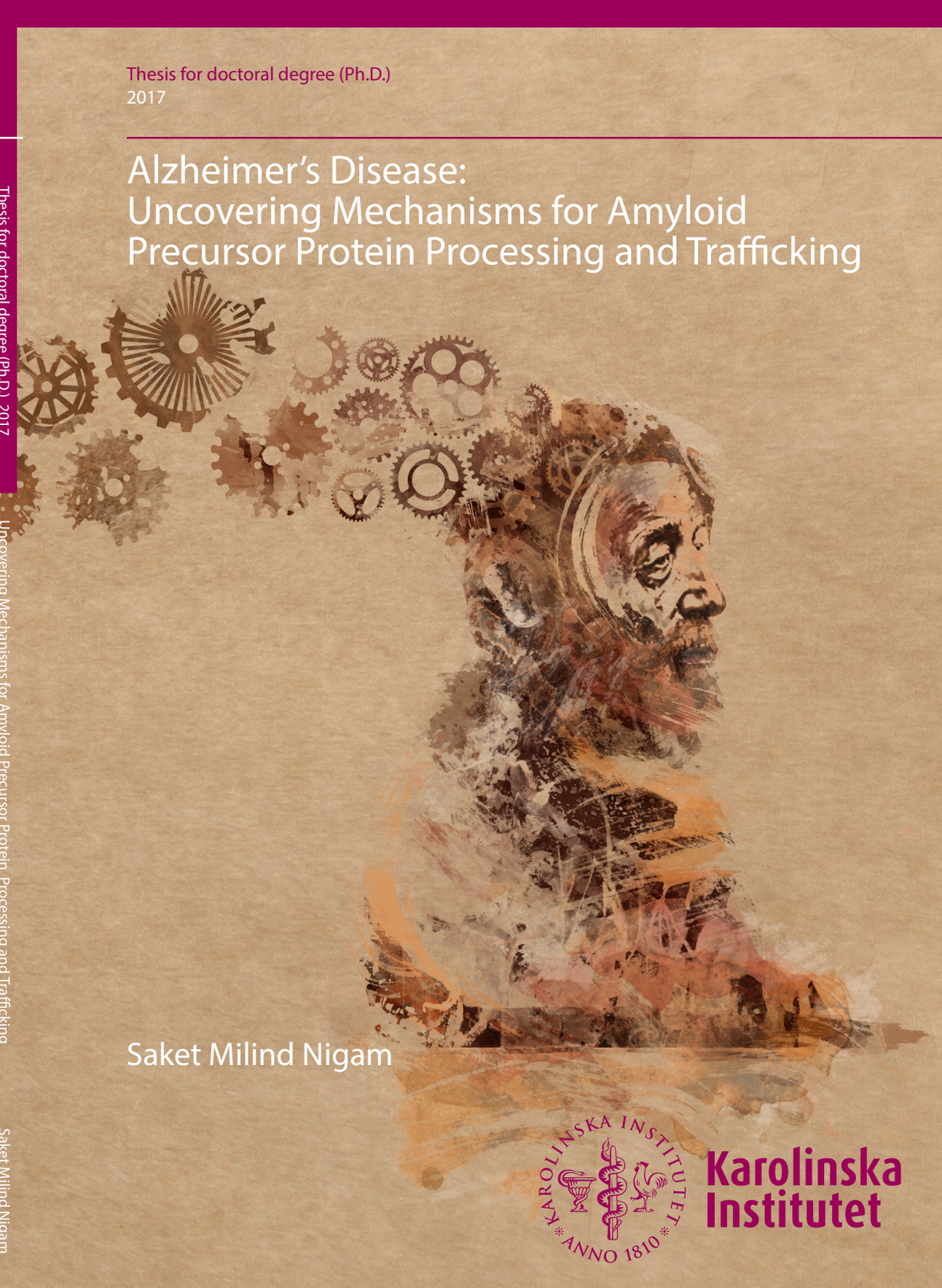

Saket Milind Nigam

Karolinska Institutet

From DEPARTMENT OF NEUROSCIENCE
Karolinska Institutet, Stockholm, Sweden

UNCOVERING MECHANISMS FOR AMYLOID PRECURSOR PROTEIN PROCESSING AND TRAFFICKING

Saket Milind Nigam

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By

Saket Milind Nigam

Principal Supervisor:
Professor Lennart Brodin
Karolinska Institutet
Department of Neuroscience

Co-supervisor(s):
Professor Mark P. Mattson
National Institutes of Health
National Institute on Aging
Laboratory of Neurosciences

Associate Professor Susanne Frykman
Karolinska Institutet
Department of Neurobiology, Care Sciences and Society
Division of Neurogeriatrics

Opponent:
Associate Professor Anna Erlandsson
Uppsala Universitet
Department of Public Health and Caring Sciences
Division of Molecular Geriatrics

Examination Board:
Associate Professor Ann-Christin Brorsson
Linköping Universitet
Department of Physics, Chemistry and Biology
Division of Chemistry

Associate Professor Taher Darreh-Shori
Karolinska Institutet
Department of Neurobiology, Care Sciences and Society
Division of Translational Alzheimer Neurobiology

Associate Professor Andrea Carmine Belin
Karolinska Institutet
Department of Neuroscience

To

Mamma, Papa and Vikrant

I love you.

॥ ॐ ॥

सरस्वति नमस्तुभ्यं वरदे कामरूपिणि ।
विद्यारम्भं करिष्यामि सिद्धिर्भवतु मे सदा ॥

Prayer to Goddess Saraswati

ABSTRACT

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by impairment of memory and, eventually, by disturbances in cognitive abilities. Brain regions crucial for learning and memory exhibit atrophy, but the underlying mechanisms for neurodegeneration continues to be point of debate. One fundamental abnormality that certainly plays a pivotal role is altered proteolytic processing of the amyloid precursor protein (APP) such that toxic amyloid-β (Aβ) peptides are formed. To prevent aggregation, enhance clearance or preclude formation of Aβ represent notable attempted strategies in the fight against AD. Because none of these strategies have - as of yet - proved triumphant, there is a persistent demand for understanding basic biological features of cells in an AD state. In this regard, this thesis collectively aims to uncover mechanisms which regulate processing and trafficking of APP and APP-relevant molecules.

Paper 1 aims to expand our understanding of endogenous APP transport to synapses of hippocampal neurons. Using immunocytochemical approaches, we find that under normal physiological conditions, APP primarily exists as cleaved fragments at nerve terminals. Perturbation of BACE1 activity, either through genetic manipulation or pharmacological inhibition, enhanced accumulation of APP at presynaptic terminals. Together with biochemical observations, this finding suggests the existence of a full-length APP trafficking pathway in neurons. Moreover, it raises questions on whether strong perturbation of BACE1 activity may be deleterious for synaptic function.

Paper 2 aims to elucidate how a protein involved in protein sorting and membrane trafficking, SNX3, may be involved in APP processing and Aβ generation. An *in vitro* cell culture model involving genetically manipulated expression of SNX3 was used in conjunction with a number of immunocytochemical techniques, flow cytometry and biochemical approaches to fulfill this aim. We found that SNX3 overexpression can perturb physical interaction of APP and BACE1 such that it results in decreased Aβ levels. This was likely the result of reduced APP internalization from the surface of cells. As such, SNX3 regulates Aβ production by influencing APP endocytosis.

Paper 3 aims to understand how exercise can lessen Aβ accumulation and how BDNF may be involved in associated regulation of APP processing. Using a transgenic mouse model of AD and cultured human neural cells, we demonstrate that exercise and BDNF can reduce

production of toxic Aβ peptides through a mechanism involving enhanced α-secretase activity. Flow cytometry, biochemical techniques and immunocytochemistry enabled us to determine that this anti-amyloidogenic APP processing involves subcellular redistribution of α-secretase and an increase in intracellular neuroprotective APP peptides capable of binding and inhibiting BACE1. Exercise, and other factors which enhance BDNF signaling, may - therefore - have both therapeutic and prophylactic potential in AD.

Paper 4 aims to determine the contribution of extracellular vesicles (EVs) to Aβ production and pathogenesis of AD. Using EVs isolated from cerebrospinal fluid and plasma of AD patients, plasma of AD mouse models and media of cultured neural cells expressing AD mutations, we determined that EVs have the capacity to destabilize neuronal Ca^{2+} homeostasis, impair mitochondrial function and sensitize neurons to excitotoxicity. Though it was found that EVs contain relatively low levels of Aβ species, the ratio between more toxic Aβ42 isoforms and Aβ40 was enhanced. The majority of this Aβ appeared to be on the surface of EVs and this appeared to be an important feature in the transcellular spread of Aβ and associated toxicity.

In summary, this thesis expands our understanding of mechanisms which regulate processing and trafficking of APP and APP-relevant molecules. In doing so, the work presented here collectively advocates for novel strategies in the fight against AD.

POPULAR SCIENCE SUMMARY

English

If moments are purely transient, then our memories are permanent. Forming an integral core of our relationships with one another, memories are part of the very essence of consciousness. Heartbreaking is the theft of these memories, and Alzheimer's Disease a menacing culprit. 200 drugs over the last 30 years have failed to solve the disease pushing scientists further and further with each passing year. Though these memories may be locked away, uplifting is the thought that a key exists somewhere out there. Herein, I present my attempts to contribute to the field of Alzheimer's Disease (AD) research with the sincere hope of finding keys for those who need it most.

The complexity of the disease has puzzled many and a litany of open questions remain to be answered. The death of cells in brain regions responsible for learning and memory are considered a critical event in AD. Though the driving force of this cell death may be unclear, the disease is characterized by the deposition of plaques in the brain. The plaques themselves are formed from a protein, called the amyloid precursor protein (APP), which can be cleaved in either a non-toxic or toxic plaque-forming fashion. To identify players which determine whether APP is destined to go down the toxic or non-toxic path is the overarching subject of this thesis.

Paper 1 lays down the foundation by examining the connections between brain cells in the context of APP cleavage. Because loss of these connections between brain cells are thought to be an important event in AD, we sought to understand whether the APP molecule is natively transported to the connection sites. **Paper 2** builds on this work by investigating a novel protein that regulates molecular transport within the cell and is capable of controlling whether APP travels down the toxic or non-toxic path. Should the protein enter the path producing toxic products, the cell has clear instructions and abilities to degrade the toxic products. In the event of dysfunctional degradation or overload of the degradation system, the toxic products can be transported out of the cell through microscopic extracellular vesicles. **Paper 4** aims to enhance our understanding of these extracellular vesicles and how they may contribute to brain cell loss in AD. Indeed, they appear to be toxic to neighboring cells. The ultimate goal of research should be to find effective treatments for patients. In this

regard, **Paper 3** proposes that exercise may be a suitable strategy because it destines APP for the non-toxic pathway.

Cumulatively, this work enhances our understanding of events involved in AD whilst providing novel approaches in the fight against it. Impossible is nothing.

Hindi

ये पल तो क्षणभंगुर हैं, मगर हमारी स्मृतियाँ स्थायी हैं. हमारे पारस्परिक सम्बन्धों का अभिन्न अंग हैं ये स्मृतियाँ और हमारी चेतना का मूलतत्व हैं. इन स्मृतियों का छिन जाना हृदयविदारक है और इसका अपराधी है Alzheimer's रोग. पिछले 30 वर्षों में लगभग 200 औषधियां भी इस रोग का निवारण करने में असफल रही हैं और हर वर्ष वैज्ञानिकों पर इस समस्या का समाधान पाने का दबाव बढ़ता जा रहा है. यद्यपि हमारी स्मृति तो स्थायी है, सुरक्षित है, और कहीं पर तो होगा इस रोग का निवारण – यही आशा है मेरी शोध की प्रेरणा का स्रोत. और इस भरोसे के साथ कि ऐसे किसी निवारण को ढूँढ़ने में कुछ योगदान दे सकूँ, अब मैं आपके सामने प्रस्तुत करूँगा Alzheimer's रोग से जुड़ी मेरी स्वयं की शोध और उसके परिणाम.

इस रोग की जटिलता ने बहुतों को हैरान किया है और बहुत से प्रश्न अभी भी अनुत्तरित हैं. Alzheimer's रोग के होने में महत्वपूर्ण भूमिका है मस्तिष्क के उन भागों की कोशिकाओं के नष्ट हो जाने की जिन पर सीखने और स्मरण रखने का दायित्व है. यद्यपि इन कोशिकाओं के नष्ट हो जाने का कारण पूरी तरह से ज्ञात नहीं है, लेकिन मस्तिष्क में एक चिपचिपे रासायनिक प्रोटीन का जमा हो जाना इस रोग का लक्षण है. इस प्रोटीन का नाम है amyloid precursor protein (APP) और यह विषैले या गैर विषैले दोनों रूप में विभाजित हो सकती है. उन तत्वों की पहचान करना, जिनसे यह निश्चित होता है कि APP का विभाजन विषैला होगा या गैर विषैला, ही इस शोध का विषय है.

पहले शोध पत्र से नींव डाली गई है मस्तिष्क की विभिन्न कोशिकाओं की पारस्परिक कड़ियों को APP के विभाजन के परिप्रेक्ष्य में समझने की. चूंकि इन कड़ियों के विच्छेद को Alzheimer's रोग में एक महत्वपूर्ण घटना माना जाता है, हमने यह समझने का प्रयास किया है कि क्या APP अणु का परिवहन इन कड़ियों से सम्बद्ध भागों तक होता है. दूसरे शोध पत्र में एक ऐसी नई प्रोटीन पर अनुसन्धान किया गया है जोकि कोशिकाओं के अन्दर आणविक परिवहन को नियन्त्रित करती है और जिसमें क्षमता है नियंत्रण करने की कि APP विषैले मार्ग पर अग्रसर होती है या गैर विषैले मार्ग पर. यदि APP अत्यधिक

मात्रा में विषैले तत्व का उत्पादन करने लगे तो उसे अति सूक्ष्म नलिकाओं के द्वारा कोशिका से बाहर निकाला जा सकता है. चौथे शोध पत्र का विषय है इन अति सूक्ष्म नलिकाओं के बारे में और ज्ञान अर्जित करना और समझना कि इनकी भूमिका मस्तिष्क की कोशिकाओं के नष्ट होने में क्या हो सकती है. ये आस-पास की कोशिकाओं के लिए विषैली प्रतीत होती हैं. इस शोध का आधारभूत लक्ष्य है रोगियों के लिए प्रभावी उपचार ढूँढ़ना. इस सन्दर्भ में, तीसरे शोध पत्र में सुझाव दिया गया है कि Alzheimer's रोग की रोकथाम में व्यायाम एक उचित रणनीति हो सकती है क्योंकि यह APP को गैर विषैले मार्ग की ओर निर्देशित करता है.

इन सभी पहलुओं को ध्यान में रखते हुए, मेरी शोध Alzheimer's रोग से जुड़ी प्रक्रियाओं के हमारे ज्ञान को और आगे बढ़ाती है और हमें इसकी रोकथाम और निवारण की दिशा में कुछ नए रास्ते दिखाती है. *वैसे भी असम्भव कुछ भी नहीं है.*

Swedish

Även om stunder är övergående, så är våra minnen permanenta. De bildar kärnan av våra relationer med varandra och är en väsentlig del av vårt medvetande. Förlust av dessa minnen är alltid sorglig och Alzheimers sjukdom är ofta skyldig. 200 olika läkemedel har under de senaste 30 åren misslyckats med att finna lösningen på denna sjukdom, vilket driver forskare längre och längre bort för varje år. Även om dessa minnen kan vara låsta är tanken att en nyckel finns någonstans där ute upplyftande. Här presenterar jag mina försök att bidra till Alzheimer (AD) forskningen med ett ärligt hopp att finna lösningar för patienterna som behöver dem mest.

Sjukdomens komplexitet har förvirrat många och ett antal öppna frågor kvarstår att besvara. Död av celler i hjärnregioner ansvariga för lärande och minne anses vara en kritisk händelse i AD. Även om skälet för denna celldöd kan vara oklar, kännetecknas sjukdomen av deponering av plack i hjärnan. Placken bildas av ett protein, som kallas Amyloid Prekursor Protein (APP), vilket kan klyvas på antingen ett icke-toxiskt eller ett toxiskt, plackbildande sätt. Att identifiera faktorer som avgör om APP är avsett att följa den toxiska eller icke-toxiska vägen är det övergripande ämnet för denna avhandling.

Artikel 1 lägger grunden genom att undersöka kopplingarna mellan hjärnceller i samband med APP-klyvning. Eftersom förlusten av dessa kopplingar mellan hjärnceller anses vara en viktig händelse i AD, försökte vi klarlägga om APP-molekylen transporteras till anslutningsställena.

Artikel 2 bygger på detta arbete genom att undersöka ett nytt protein som reglerar transporten av molekyler inom cellen och kan kontrollera om APP tar den toxiska eller icke toxiska vägen. Om proteinet kommer in på vägen som producerar toxiska produkter, har cellen tydliga instruktioner och kapacitet att bryta ner dessa produkter. I händelse av dysfunktionell nedbrytning eller överbelastning av nedbrytningssystemet kan de toxiska produkterna transporteras ut ur cellen via mikroskopiska extracellulära vesiklar. **Artikel 4** syftar till att förbättra vår förståelse av dessa extracellulära vesiklar och hur de kan bidra till hjärncellsförlust i AD. Faktum är att de verkar vara toxiska för närliggande celler. Det ultimata målet med AD forskning bör vara att hitta effektiva behandlingar för patienter. I detta avseende föreslår **artikel 3** att träning kan vara en lämplig strategi eftersom den kan driva APP att klyvas via den icke-toxiska vägen.

Kumulativt förbättrar detta arbete vår förståelse av händelser som är inblandade i AD samtidigt som det indikerar nya tillvägagångssätt i kampen mot denna sjukdom. Inget är omöjligt.

LIST OF SCIENTIFIC PAPERS

I. **Nigam S. M.**, Xu S., Ackermann F., Gregory J. A., Lundkvist J., Lendahl U., Brodin L. (2016) Endogenous APP accumulates in synapses after BACE1 inhibition. *Neurosci. Res*. 109, 9–15.

II. Xu S., **Nigam S. M.**, Brodin L. Overexpression of SNX3 decreases amyloid-β peptide production by reducing endocytosis of amyloid precursor protein. *Manuscript.*

III. **Nigam S. M.**, Xu S., Kritikou J. S., Marosi K., Brodin L., Mattson M. P. (2017) Exercise and BDNF reduce Aβ production by enhancing α-secretase processing of APP. *J. Neurochem.* 38, 42–49.

IV. Eitan E., Hutchison E. R., Marosi K., Comotto J., Mustapic M**., Nigam S. M.**, Suire C., et al. (2016) Extracellular vesicle-associated Aβ mediates trans-neuronal bioenergetic and Ca2+-handling deficits in Alzheimer's disease models. *Npj Aging Mech. Dis.* 2, 16019.

CONTENTS

LIST OF ABBREVIATIONS

AD	Alzheimer's disease
ADAM	A disintegrin and metalloproteinase
ADP	Adenosine diphosphate
AICD	Amino-terminal APP intracellular domain
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
APLP	APP-like proteins
APOE	Apolipoprotein E
APP	Amyloid precursor protein
ARF6	GTPase ADP ribosylation factor 6
Aβ	Amyloid β
BACE1	β-site amyloid precursor protein cleaving enzyme 1
BBS	α-bungarotoxin-binding site
BDNF	Brain derived neurotrophic factor
BiFC	Bimolecular fluorescence complementation
CaMK2	Ca2+/calmodulin-dependent protein kinase II
CNS	Central Nervous System
CSF	Cerebrospinal fluid
CTF	C-terminal fragment
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
ER	Endoplasmic reticulum
EV	Extracellular vesicle
FAD	Familial Alzheimer's disease
FDA	Food and Drug Administration
GGA	Golgi-localized, gamma adaptin ear-containing, ARF-binding
GTPase	Guanosine triphosphate hydrolase
HEK293T	Human embryonic kidney cells 293T
IL-1	Interleukin-1
LTD	Long-term depression
LTP	Long-term potentiation
MAPK	Mitogen-activated protein kinase
miRNA	Micro ribonucleic acid
mRNA	Messenger ribonucleic acid
MVB	Multivesicular bodies
NMDA	N-Methyl-D-aspartic acid
PET	Positron emission tomography
PLA	Proximity ligation assay
ROS	Reactive oxygen species
rRNA	Ribosomal ribonucleic acid
sAPP	Soluble amyloid precursor protein
SNX	Sorting Nexin
SV2	Synaptic vesicle glycoprotein 2A
TGN	Trans Golgi network

1 INTRODUCTION

The quest for extending longevity has captivated the imagination of mankind - markedly unrestricted by cultural boundaries - over the last several millennia. These anti-aging ambitions can be illustrated by the development of innumerable myths on how we can achieve immortality. Indeed, the 'Fountain of Youth', 'Elixir of Life', religious nirvana and snake oil are just a small subset of our collective efforts. Though inspiring, these mythological potions and practices proved fruitless as the average life expectancy generally remained below 40 years of age for much of human history. Our big breakthrough came instead by way of advances in healthcare and sanitation in the 20th century. While the ensuing explosion in worldwide life expectancy amounts to a current figure crossing the 70 year mark, this trend is not without some caveats. Generally, our ancestors rarely suffered from conditions like heart disease, cancer and loss of mental function - conditions which are all too common presently. It seems, thusly, that while we have pushed the bounds of human longevity we have also ushered in a basket of new challenges. Alzheimer's Disease - being intimately linked to aging - is one such condition that has and will continue to test scientists, caregivers and families as we voyage into the 21st century. Here I present my scientific efforts to contribute to the ever-expanding Alzheimer's Disease research field with the ultimate goal of aiding individuals and families afflicted by this devastating disease.

1.1 ALZHEIMER'S DISEASE

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder that leads to a variety of symptoms that are heavily relied upon for diagnosis. The symptoms - which are also progressively detrimental in nature - begin with memory problems, confusion and reduced concentration, and can eventually manifest in the form of severe memory deficits, difficulties in speaking, motor impairment, anxiety, paranoia and other cognitive disabilities. In the final stages of the disease, patients are completely reliant on caregivers for management of both their physical and mental well-being. The costs associated with caregiving as well as medicinal treatment are projected to pose a great challenge to society as our global population ages and prevalence of the disease increases. Indeed, the US-based Alzheimer's Association estimates that around 5 million Americans are living with the condition presently (in 2017) amounting to over $250 billion in care costs. By 2050, the organization predicts that both of these figures will more than triple in the US alone. This threat reverberates across the globe. By 2050, over 150 million individuals worldwide are expected to face the progressive and ultimately fatal

condition[1]. With such dire predicted outcomes and an absence of effective treatments, governments, research institutions, healthcare providers and biopharmaceutical companies are mounting a concerted effort to understand and manage the disease[2].

1.2 NEUROPATHOLOGICAL BASIS OF AD

1.2.1 History

In 1906, the world was introduced to the case of Auguste Deter. Physician Alois Alzheimer described his 50-year old female patient as experiencing progressive sleep and memory disturbances, aggression and confusion. The report initially generated little interest among psychiatrists and neuropathologists of the time, but Alzheimer persisted in his characterization of the peculiar condition. After an autopsy of the patient was conducted, Alzheimer managed to perform both morphological and histological investigations of Deter's brain. In addition to general brain atrophy, he noted alterations that would later be described as senile plaques and neurofibrillary tangles. Together, these alterations would constitute the classical hallmarks of Alzheimer's Disease. For much of the 20th century, the Alzheimer name remained in relative obscurity until a resurgent prevalence of the disease prompted renewed interest in investigating its neuropathological basis.

Our understanding of AD has since expanded to incorporate the notion that AD is a multifactorial disease. In addition to plaques and tangles, patients may be plagued with a number of pathologies including microgliosis, astrocytosis, neuritic dystrophy, neuronal loss and synaptic insufficiencies[3]. The search for a key regulator and comprehensive hypothesis of these events began in the 1980s - ultimately leading to the development of the "amyloid cascade hypothesis"[4].

1.2.2 The Amyloid Cascade Hypothesis

Much of the AD research community has focused on senile plaques as the key player in pathogenesis. In 1984, the Amyloid-β (Aβ) protein was identified to be the core constituent of the senile plaques[5]. The ensuing "amyloid cascade hypothesis" became the dominant theory for the mechanism of AD pathogenesis. The hypothesis holds that Aβ is the causative agent in AD and lies upstream to other observed pathologies like neurofibrillary tangles, cell loss, vascular damage and dementia[4]. Aβ peptides can range from 38 to 43 amino acids in length[6]

with the most abundant species being Aβ40 and Aβ42. Importantly, Aβ42 has a hydrophilic structure which makes it particularly prone to aggregation and subsequent plaque deposition[7]. As such, an imbalance in Aβ production favoring the Aβ42 form is viewed as a marker for toxicity. While our understanding of this protein has expanded considerably over the last 30 years, a complete picture remains enigmatic. As a result, the amyloid cascade hypothesis has been challenged based on the following observations:

- Amyloid plaque burden is a relatively poor correlate of cognitive impairment
- Aβ deposits can be found in post-mortem brain of non-demented individuals
- Neurofibrillary tangles can be observed prior to plaque deposits

Notably, these challenges focus on plaque deposits and overlook the contribution of diffuse plaques and/or pre-plaque forms of Aβ (e.g. oligomeric Aβ). A comprehensive point/counter-point can be reviewed elsewhere[8], but it is important to note that several lines of evidence indicate that Aβ is tightly linked to the degeneration observed in AD:

- Genetic mutations resulting in altered Aβ levels are invariably connected to AD (discussed in section 1.4)
- Aβ42 oligomers can decrease synapse density, inhibit LTP, enhance LTD and impair memory
- Aβ oligomers can induce hyperphosphorylation of tau

These and other lines of evidence cumulatively provide a strong basis for continued research within the realm of the amyloid cascade hypothesis. Accordingly, the present thesis focuses on upstream events that precede plaque formation - namely the proteolytic processing of the Amyloid Precursor Protein (APP), which can produce Aβ.

1.3 AMYLOID PRECURSOR PROTEIN

Discovered in 1987[9] as a precursor to Aβ, the Amyloid Precursor Protein (APP) is a type-1 transmembrane protein with a large N-terminal domain, a single transmembrane region and a short C-terminal domain. The protein belongs to a gene family that is evolutionarily conserved across multiple species including *C. elegans*, *drosophila melanogaster* and mammals. While multiple isoforms of mammalian APP have been identified (e.g. 695-, 751-, 770-amino acids) and are ubiquitously expressed, there appears to be a preference for specific isoforms across

cell types. Cells of neuronal origin, for instance, express primarily the 695-amino acid isoform[10]. The normal functions of APP are not fully understood, but evidence suggests a role in neuronal survival, neurite outgrowth, synaptic plasticity and cell adhesion[11].

Importantly, there are also two APP homologues, termed APP-like proteins, that are expressed in the nervous system: APLP-1 and APLP-2. Both share domain structure similarity with APP and appear to have some redundant functions. Transgenic mice with single knockouts of either APP(-/-)[12], APLP-1(-/-)[13] or APLP-2(-/-)[14] do not exhibit any severe phenotypes. Interestingly, mice with double-knockouts of APP(-/-)/APLP-1(-/-) are viable, but APP(-/-)/APLP-2(-/-) knockout is lethal. As such, there may be a key role for APLP-2 in embryonic development[13]. Moreover, proteolytic processing of APLP-1 and APLP-2 can occur by the same secretases which process APP and result in similar products[15] (although only APP is capable of generating an amyloidogenic fragment[16]). These findings naturally complicate the process of finding a treatment for AD as non-APP targets must be considered during the drug development process.

1.3.1 Amyloid Precursor Protein Processing

In neuronal cells, two distinct pathways - termed the non-amyloidogenic and amyloidogenic pathways - compete for APP as a substrate. In the former pathway, an initial cleavage of APP by α-secretase liberates a soluble peptide, APPsα (henceforth referred to as sAPPα). The remaining membrane-bound α-CTF fragment is further processed by γ-secretase to generate non-toxic P3 and AICD fragments. Conversely, the amyloidogenic pathway for APP processing involves an initial cleavage by β-secretase liberating a soluble peptide, APPsβ (henceforth referred to as sAPPβ). γ-secretase further processes the remaining β-CTF fragment to produce the toxic Aβ peptides along with AICD fragments (Figure 1). Though not discussed

in the present thesis, there may be additional APP processing pathways including one recently identified to involve η-secretase[17].

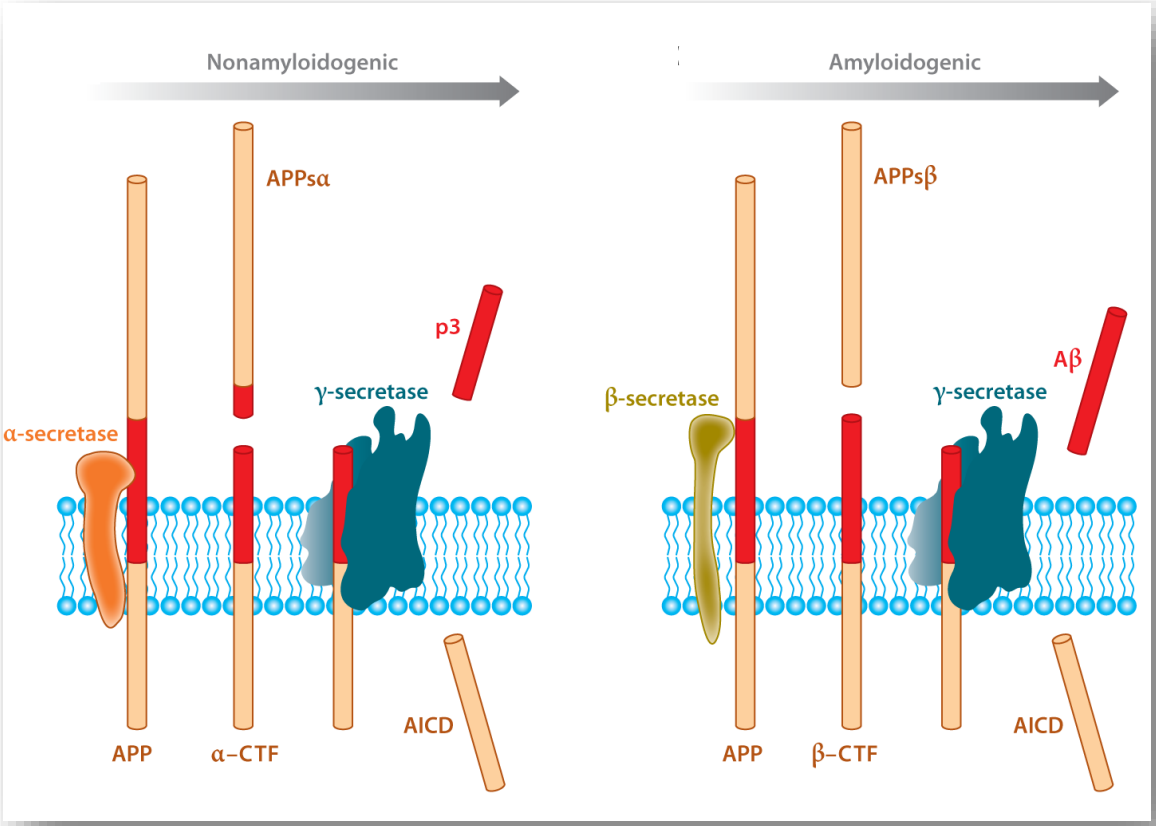

Figure 1. Illustration of non-amyloidogenic and amyloidogenic pathways for APP processing.[16]

The physiological functions of APP-derived peptides are emerging rapidly and provide us with a more complete picture of the potential consequences of manipulating the APP processing pathways.

1.3.1.1 sAPPα

sAPPα is generated by α-secretase mediated cleavage of APP. The peptide is generally prescribed to be beneficial as it can protect neurons from oxygen-glucose deprivation and excitotoxicity[18]. Moreover, sAPPα promotes neurite outgrowth, synaptogenesis, cell adhesion and LTP[11,19,20] as well as learning and memory in rodents when administered intracererebroventricularly[21,22]. Recent evidence further indicates the sAPPα can directly bind to and inhibit β-secretase cleavage of APP[23]. This finding suggests the existence of an endogenous positive feedback loop that can promote the non-amyloidogenic pathway over the amyloidogenic pathway for APP processing.

In patients genetically predisposed to developing AD, low levels of sAPPα in the cerebrospinal fluid (CSF) are observed and this correlates with poor performance on neuropsychological tests[24]. Although patients who develop AD sporadically have unaltered levels of sAPPα in their CSF[25], there is a strong cumulative basis for enhancing sAPPα as a strategy for treatment of AD. However, owing to its large size, sAPPα is unable to cross the blood brain barrier effectively. Thus, increasing α-secretase activity is seen as the most direct and reasonable approach to increasing sAPPα levels[26].

The molecular identity of α-secretase remained elusive until 2010 when it was reported that ADAM10 is the main physiologically relevant α-secretase[27]. A quest for ADAM10 activators has begun, but it is important to remember that ADAM10 has multiple substrates including epidermal growth factor receptor ligands. Thus, strong widespread activation of ADAM10 may lead to tumor growth[28]. Given the existence of the aforementioned positive feedback loop involving sAPPα[23], hyperactivation of ADAM10 may not be necessary in treatment of AD.

1.3.1.2 P3

The non-amyloidogenic processing of APP also liberates a P3 fragment (Figure 1). No clear biological role has been established for this fragment[29].

1.3.1.3 AICD

The amino-terminal APP intracellular domain (AICD) is liberated after γ-secretase processing of APP in either the non-amyloidogenic or the amyloidogenic pathway (Figure 1). The resulting fragment is approximately 57-59 amino acids in length, but additional cleavage by other enzymes can lead to shorter fragments ranging from 31-50 amino acids in length[29]. AICD is thought to interact with proteins such as Fe65, translocate to the nucleus and function as a transcriptional activator of several genes (e.g. p53). Much is still unknown regarding the functions of AICD in the context of AD, but a comprehensive review can be found elsewhere[30].

1.3.1.4 sAPPβ

The sAPPβ fragment is generated following β-secretase cleavage of APP (Figure 1) and appears to lack the neurotrophic effects associated with sAPPα[31]. Rather, it was linked to

disintegration of axons and pruning of synapses during development. sAPPβ performs this function by acting as a ligand to death receptor 6 and initiating caspase 6 signaling. Interestingly, withdrawal of neurotrophins induces production of sAPPβ and subsequent degeneration[32].

In APP(-/-)/APLP2(-/-) double knock-out mice, sAPPβ does not prevent perinatal lethality[33]. This is in contrast with sAPPα knock-in which results in viable double knock-out mice[34]. As such, the extra 16 amino acids at the C-terminus of sAPPβ point to key functional differences as compared to the shorter sAPPα fragment. These contrasting effects provide further motivation to pursue strategies that shift processing of APP towards the non-amyloidogenic pathway.

1.3.1.5 Amyloid β

Sequential cleavage by β-secretase and γ-secretase liberates Aβ peptides of varying lengths (Figure 1). The Aβ monomers can come together to form neurotoxic oligomers prior to formation of Aβ fibrils and amyloid plaque deposits. While Aβ40 is the predominant length produced via APP processing, Aβ42 is more prone to aggregation. This propensity for Aβ oligomers to aggregate have placed the peptide at the center of AD research. The current prevailing thought is that chronic imbalance in the production of Aβ relative to its clearance underlies the observed neurotoxicity in AD. Although the mechanisms for neurotoxicity are not fully understood, one hypothesis states that Aβ oligomers in the extracellular space can inhibit NMDA-mediated synaptic transmission and ultimately promote synapse loss[3]. This hypothesis has been further adapted to include oligomeric Aβ's relationship to a number of receptors including AMPA receptors, LRP1 protein, α7 receptors and RAGE receptors[35]. As such, Aβ levels have been one of the most commonly used functional readouts in AD research. Importantly, although Aβ may be a valuable biomarker for clinical diagnosis, levels in the CSF are actually reduced in AD patients - not increased[36]. This likely reflects reduced clearance of Aβ through the CSF and, accordingly, accumulation in the brain.

An increasing amount of attention is being given to the normal physiological functions of Aβ. Monomeric Aβ, for example, has neurotrophic and neuroprotective effects, can promote proliferation of neural progenitor cells and may be critical for synaptic function. These proposed functions are largely true when Aβ is present at low concentrations precluding formation of Aβ oligomers[35]. Therefore, delicate manipulation of APP processing pathways

may be a more advisable treatment strategy than strong perturbation of the amyloidogenic pathway.

1.3.2 Subcellular Trafficking of APP

The cellular trafficking of APP is heavily implicated in its processing. Under the canonical model (Figure 2), APP is synthesized in the endoplasmic reticulum and is transported to the Golgi Apparatus/Trans-Golgi Network (TGN) where it undergoes posttranslational modifications. The TGN is the major site of resident APP with just 10% of APP molecules being further transported to the plasma membrane via secretory vesicles[37]. APP which reaches the plasma membrane can be processed by α-secretase to generate sAPPα[38]. APP which is not processed and shed at the plasma membrane is internalized within minutes due to the presence of a "YENPTY" internalization motif at the C-terminus via clathrin-dependent endocytosis[39]. This internalization presents APP to endosomes where it can undergo β-secretase mediated processing and generate Aβ. Accordingly, inhibition of endocytosis can prevent β-secretase processing of APP[40]. Typically, increased delivery of APP to the surface or reduced internalization favors the non-amyloidogenic pathway for processing while enhanced retention in endosomes favors the amyloidogenic pathway for processing[41].

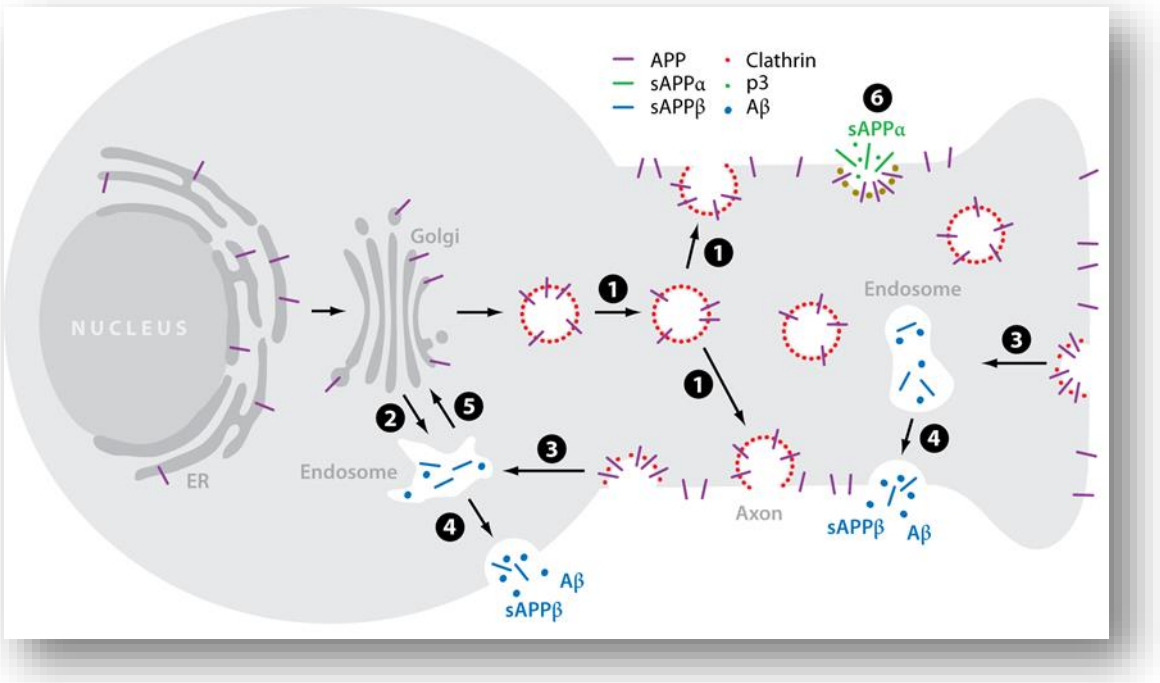

Figure 2. Illustration of the canonical APP trafficking model. APP leaves the Golgi and traffics down the axon (1) or, alternatively, traffics into an endosomal compartment (2). After insertion at the cell surface, some APP is re-internalized into endosomes (3) where Aβ is produced. Endosomes can then recycle to the cell surface expelling Aβ (4). Alternatively, APP can also be transported from endosomes back to the Golgi (5). APP which is not internalized is subject to α-secretase cleavage at the cell surface expelling sAPPα (6).[16]

Once at endosomes, APP/APP-derived peptides (including Aβ) can be trafficked through other endocytic and recycling organelles to the Trans-Golgi Network, the cell surface or lysosomes for degradation[37].

1.3.3 Extracellular Vesicles

It has been proposed that when the accumulation of Aβ is beyond the clearance capacity of lysosomes or glial cells, Aβ may be released into extracellular space and spread through the brain via extracellular vesicles[42]. Extracellular vesicles, including exosomes, are small vesicles (50-250 nm in diameter) which can be released from most cell types (including in the CNS) and participate in elimination of cellular waste, regulating immune responses and communicating between neural cells[43–45]. Notably, they can carry molecular content over long distances via bodily fluids. There are several categories of EVs based on their secretion pathways[46,47]:

- Apopotosomes are released from cells undergoing apoptosis
- Microvesicles can be relased by evagination of the plasma membrane
- Exosomes can be released via fusion of multivesicular bodies (MVB) with the plasma membrane.

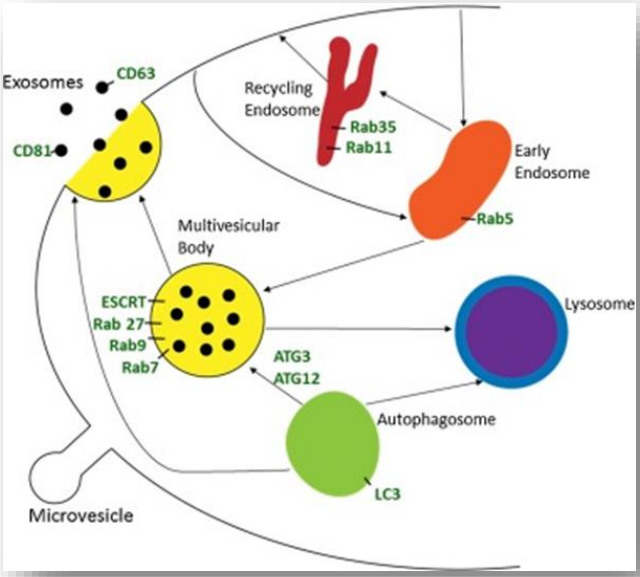

Figure 3. Subcellular pathways for the packaging, lysosomal degradation and vesicular extrusion of damaged and aggregated proteins.[46]

Current methodologies are unable to distinguish between these categories and so they are collectively referred to as the EVs[48]. Because exosomes are released from MVBs, they are also a part of the endosomal-lysosomal system (Figure 3). As such, the molecular contents of exosomes commonly consist of proteins that may have an endosomal past including tetraspanins, Rab GTPases, flotilin,

Alix, TSG101, heat shock proteins[49–52] as well as a variety of genetic material (e.g. DNA, mRNA, miRNA, rRNA)[53–55]. Although it is not clear what drives MVBs to fuse with plasma membranes (as opposed to trafficking cargo to lysosomes for degradation), inhibition of the lysosome pathway (e.g. with Bafilomycin A) enhances exosome secretion at the plasma membrane[56]. This finding suggests that EVs may function as an alternative disposal pathway to compensate for lysosomal dysfunction or in the event of misfolded protein overload[57].

Indeed, both Aβ and phosphorylated Tau - representing pathologically misfolded proteins in AD - can be found in EVs isolated from CSF, brain extracellular space and blood samples from AD patients[58–60]. Because these proteins have been demonstrated to inhibit autophagy flux and lysosomal function[61], their release via exosomes may indicate a compensatory handling mechanism. In addition to handling intracellular Aβ, EVs have the ability to interact with and bind to Aβ extracellularly and thereby facilitate Aβ uptake by microglia[62]. These findings, cumulatively, provide support for the possibility of using EVs as biomarkers in disease diagnosis and treatment evaluation. Importantly, it is not clear whether EVs attenuate neurodegeneration or whether they can promote it. The notion and corresponding evidence that EVs may contribute to a prion-like spreading of Aβ[63–65] is currently counterbalanced with evidence that EVs act as scavengers of Aβ[66–68]. Therefore, expanding our understanding of how EVs may be involved in extracellular aggregation and spread of Aβ could also aid in the development of AD therapeutics. **Paper 4** of the present thesis aims to enhance our understanding of the contribution that EVs play in Aβ production and pathogenesis of AD.

1.3.4 Synapses

Neurons, as highly specialized cells, rely on synapses to propagate signals between cells. As such, synapse loss is expected to perturb communication across brain networks. In the context of AD, synapses are sites of early pathological changes and loss of synapses correlates with cognitive deficits[69]. The cause of this synaptic impairment is unclear, but proteolytic processing of APP is likely involved[37] since synapses appear to be a major site for secretion of Aβ[70,71] and oligomeric Aβ can dose-dependently decrease synaptic function, the number of synapses and impair memory[72]. Moreover, both β- and γ-secretase are present at synaptic terminals[73,74]. Despite this awareness, it remained unclear whether APP is trafficked to synapses for local processing or if the processing occurs elsewhere in neurons. This open question served as the motivation for **Paper 1** of this thesis.

1.3.5 SNX Family of proteins

Because endosomal trafficking and protein sorting (both at synapses as well as somatic compartments) is intimately connected to APP processing, a growing avenue of research aims to identify novel regulators for APP trafficking. Among these, several members of the sorting nexin (SNX) family have been demonstrated to regulate Aβ generation. SNXs are cytosolic or membrane-associated proteins which can be characterized by the presence of a phox-homology domain and a variable number of protein-protein interaction domains[75,76]. Collectively, they have been demonstrated to play key roles in the trafficking of protein cargo. Both positive and negative regulators of amyloidogenic processing of APP have been found among the SNX family. For example, SNX33 promotes α-secretase cleavage of APP[77] while SNX27 influences Aβ generation by controlling the assembly of γ-secretase[78]. While there are over 30 members in the SNX family[79], only a handful have been connected to APP processing. **Paper 2** in this thesis provides evidence that a novel regulator, SNX3, can influence Aβ levels by altering APP internalization.

1.3.6 Subcellular Trafficking of APP-relevant Secretases

As the co-distribution of APP with α-, β- and γ-secretases dictates the nature of APP processing, considerable research has been conducted to understand trafficking of APP-relevant secretases as well.

1.3.6.1 α-secretase

In neural cells, α-secretase processing of APP is often connected with its localization at the plasma membrane[80]. However, it is also present and catalytically active in the TGN. The difference between these two processing sites may be related to constitutive versus regulated α-secretase activity. The latter has been shown to occur in the TGN in response to stimulation[81] and can lead to α-secretase activity beyond its constitutive level. For most α-secretase stimulators, it is is not clear whether they affect ADAM10 or other proposed α-secretases (e.g. ADAM9, ADAM17)[82]. The phorbol ester phorbol myristate acetate, for example, requires ADAM17 to activate α-secretase[27] while the neuropeptide pituitary adenylate cyclase-activating polypeptide stimulates α-secretase through ADAM10[83]. Activation of α-secretase through stimulators is primarily thought to occur via different signaling pathways including the

protein kinase C and MAPK pathways[84]. However, this underscores the possible contribution of α-secretase trafficking in regulation of its activity. Though little is known regarding the underlying mechanisms, α-secretase trafficking can - indeed - be controlled. Tetraspanins and nardilysin, for example, have been implicated in ADAM10 trafficking[82]. In primary neurons, short-term NMDA activation drives ADAM10 to the postsynaptic membrane through interaction with synapse-associated protein 97[85]. Moreover, an endoplasmic reticulum retention signal on the C-terminus of ADAM10 has now been identified to regulate its transport to the plasma membrane. These studies cumulatively suggest that trafficking of α-secretase to distinct subcellular locations may affect its catalytic efficiency.

1.3.6.2 β-secretase

It is well accepted that BACE1 is the main catalytically active β-secretase responsible for cleaving APP[86]. As such, it has been an attractive target for AD therapy. Indeed, BACE1 levels and activity are increased in the AD brain[87] and downregulation of BACE1 in mice can inhibit Aβ generation[88–91]. It is synthesized in the ER and subsequently transported to the Golgi apparatus where it undergoes maturation. There, the pro-domain of BACE1 is removed by furin leading to a ~2-fold increase in activity[92]. BACE1 can then be transported to the plasma membrane or endosomal compartments. It is important to remember that BACE1 is rapidly internalized from the cell surface[93], is predominantly intracellular[89] and is active in acidic microenvironments[94]. Therefore, endosomes are viewed as the main β-secretase processing site for APP. Several proteins have been identified to regulate BACE1 trafficking. The GGA family of monomeric clathrin adaptor proteins, for example, can traffic BACE1 from endosomes. Phosphorylation within the DISL motif - at the cytoplasmic domain of BACE1 - promotes retrieval to the TGN[95] whereas non-phosphorylated BACE1 is sent to the plasma membrane[96]. The sorting nexin (SNX) family of proteins are also involved in BACE1 trafficking. SNX6 interaction with BACE1 retains the secretase in the endosome preventing transport to the TGN[97] while SNX12 has been shown to regulate BACE1 trafficking between the cell surface and endosomes[98]. Interestingly, the endocytosis of APP and BACE1 appear to be spatially distinct involving a different class of endocytic vesicles. In contrast to APP, which is internalized through clathrin-dependent endocytosis, BACE1 is sorted from the plasma membrane to endosomes via a route controlled by the GTPase ADP ribosylation factor 6 (ARF6)[99]. As a result, targeting distinct endosomal routes may be one valuable way to regulate BACE1 processing of APP. Indeed, an endosomally-targetted, sterol-linked BACE1 inhibitor took advantage of this differential subcellular compartmentalization and specifically inhibited

amyloidogenic processing of APP while permitting BACE1 processing of non-amyloid substrates[100].

1.3.6.3 γ-secretase

Following cleavage by α- or β-secretase, APP can be further processed by γ-secretase. Presenilin, nicastrin, anterior pharynx-defective 1 and presenilin enhancer 2 come together to form a functional γ-secretase complex. Synthesis of the individual components and formation of the overall complex takes place in the ER and Golgi compartments[101,102]. Mature γ-secretase can then be transported to the plasma membrane where it can remain or be internalized into endosomes, multivesicular bodies or the lysosome[103]. Like both α- and β-secretase, γ-secretase trafficking relies on its interaction with a number of transport proteins. These proteins include Rab protein family members, PLD1, ARC, β2-adrenergic receptor, GPR3, β-arrestin 2, RER1[41]. To discuss the relationship between all of these proteins and the various components of γ-secretase is beyond the scope of the presented thesis, but a comprehensive review[103] can be consulted for further information.

1.4 GENETICS

The amyloid cascade hypothesis for AD pathogenesis is largely substantiated by a number of genetic studies. The APP gene is located on chromosome 21 and triplication of this chromosome - as is seen in Down's Syndrome patients - yields three copies of the APP gene. This enhances APP expression and Aβ accumulation. Indeed, patients with Down's Syndrome experience an early onset of AD pathology[104]. In addition to triplication of chromosome 21, inherited familial Alzheimer's Disease (FAD) mutations in APP can cause early-onset Aβ deposition[105]. Most of these mutations are located near the α-, β- and γ-secretase cleavage sites and can be examined in depth on the Alzheimer Disease and Frontotemporal Dementia Mutation Database (http://www.molgen.ua.ac.be/ADmutations/). Importantly, a mutation in APP was discovered that appears to protect against AD and age-related cognitive decline. The mutation, A673T, is located near the BACE1 cleavage site and impairs BACE1 cleavage of APP[106]. This finding further supports the amyloid hypothesis of AD pathogenesis. Mutations in the presenilin gene have also been described and constitute the most severe forms of AD with complete penetrance and onset occurring as early as 30 years of age[107]. The mutations tend to alter the transmembrane domains of the PS1 and PS2 proteins and, as is the case with

APP mutations, these mutations have the shared effect of increasing the levels of Aβ42 relative to Aβ40[108].

Importantly, the aforementioned mutations in APP and presenilin are heavily implicated in early-onset AD (defined as occurring prior to 65 years of age), but this only represents ~1% of all AD cases. The majority of cases are late-onset and typically sporadic - involving no family history of AD[107]. The genetic basis of sporadic forms of AD remains enigmatic despite multiple genome wide association studies and attempts to identify novel risk factors. The best established risk factor, APOε4, is present in about 50% of late-onset AD patients[109]. Although carriers of two copies of the allele have 12 times the risk of developing late-onset AD compared to non-carriers (single copy carriers have 3 times the risk), APOε4 is neither necessary nor sufficient for development of AD thereby complicating its diagnostic utility[110]. Moreover, the mechanism by which APOε4 affects the risk of AD is not fully understood, but it is suggested to be involved in conversion of monomeric Aβ to more toxic, aggregative forms as well as in clearance of Aβ from the brain[111].

Although a number of additional risk genes have been identified for late-onset AD, they have weaker association to the disease as compared to APOε4. Taken together, there is likely a complex interplay between genetic and environmental factors which underlies development of sporadic forms of AD.

1.5 ALTERNATIVES TO THE AMYLOID CASCADE HYPOTHESIS

The "amyloid cascade hypothesis" described above in section 1.2.2 is the most popular hypothesis for AD-related neuropathologies and cognitive impairments. It holds that all other pathologies observed in patients are downstream events of excessive accumulation of Aβ. Although the presented thesis and constituent articles are built upon this hypothesis, it is important to acknowledge that multiple hypotheses for AD have been proposed. A few of these hypotheses are discussed briefly in this section.

1.5.1 Tau Hypothesis

The tau hypothesis is perhaps the most heavily investigated among the alternatives to the amyloid cascade hypothesis. In addition to senile plaques, neurofibrillary tangles were observed in the original characterization of AD brain over a century ago. However, the

molecular precursor to these tangles (i.e. hyperphosphorylated forms of the protein tau) was determined much later - in the 1980s[112]. Subsequently, a "tau hypothesis" was generated positing that tau phosphorylation and aggregation is the common denominator for the multitude of deleterious observations found in AD. The hypothesis draws support from both neurobiological studies[113] as well clinical data[114,115]. Importantly, hyperphosphorylated tau in the brain and CSF correlates with the severity of dementia[116,117] while Aβ plaques are considered a relatively poor correlate[118]. Tau, as a major component of the microtubule-associated proteins, plays a critical role in stabilizing microtubules in axons. Hyperphosphorylation of tau is thought to disrupt this process ultimately leading to neurotoxicity[119,120]. The details of this mechanism are beyond the scope of this thesis, but can be reviewed elsewhere[121].

1.5.2 Oxidative Stress Hypothesis

Oxidative stress refers to the imbalance between formation of reactive oxygen species (ROS) and the cell's ability to clear them. Formed during oxidative phosphorylation by the electron transport chain in mitochondria, ROS interacts with a number of macromolecules. Excessive levels of ROS can place cells in a state of oxidative stress and lead to damage of the cell and its organelles. One prominent theory suggests that ROS is at the center of the normal aging process[122]. This theory extends to the AD research field as AD patients have high levels of oxidative damage[123–126]. ROS can have widespread effects on cells including to induce modifications to proteins. Such modifications may have the ability to promote neurodegeneration via protein misfolding[127]. It is not clear what causes the ROS imbalance observed in AD, but reviews can be consulted for in-depth characterization of molecular interaction partners and mechanisms[126].

1.5.3 Inflammation Hypothesis

Presence of pro-inflammatory cytokines and activated complement factors has led to the idea that neuroinflammation is involved in the pathology of the disease. Though typically considered a downstream event to the amyloid hypothesis (with Aβ causing activation of microglia), neuroinflammation may exacerbate the course of disease[128]. On the other hand, polymorphisms in genes related to regulation of inflammatory processes offer a compelling explanation for sporadic forms of AD. IL-1 polymorphisms, for example, have been associated with differing degrees of microglial activation[129]. Moreover, APOε4 carriers with AD have

more microglial activation. Importantly, the mechanism of action for AD pathogenesis by microglial activation is still unclear, but epidemiological studies indicate that individuals receiving long-term anti-inflammatory therapy have a lower prevalence of AD[130].

1.5.4 Other Hypotheses

In addition to the prominent hypotheses described above, vascular[131], cholesterol[132], metal[133] and cell cycle[134] hypotheses have been proposed. To discuss all of these is well beyond the scope of this thesis, but the existence of so many hypotheses illustrates an important point. Namely that AD is a multifactorial disorder involving a litany of pathological events. As such, effective treatments will have to account for the diversity in events that precede neurodegeneration.

1.6 TREATMENT STRATEGIES

To date, only symptomatic medications have been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for treatment of AD. These include acetylcholinesterase inhibitors (e.g. donepezil, galantamine, rivastigmine) and a partial NMDA receptor antagonist (i.e. memantine). Acetylcholinesterase inhibitors are used to elevate the level of acetylcholine at synapses counteracting the loss of cholinergic function and neurotransmission found in AD. Memantine, on the other hand, restricts glutamate-induced toxicity[135]. Under normal physiological conditions, NMDA receptors are blocked by magnesium ions. Strong glutamate signaling depolarizes the post-synaptic membrane and reverses the NMDA block allowing calcium ions to enter the postsynaptic neuron. In AD, however, constant glutamate stimulation and NMDA receptor over-activation is observed leading to dysfunction. Thus, memantine is thought to prevent excitotoxicity caused by excessive glutamate stimulation[136]. While these therapies have been shown to improve symptoms and may arrest the rate of cognitive decline, they do not prevent decline or reverse the neuronal damage seen in AD[137].

In light of results from genetic studies and the large body of evidence supporting the amyloid cascade hypothesis, the most attractive disease-modifying approaches have been to either disrupt aggregation, promote removal or prevent formation of Aβ. Compounds including glycosaminoglycan 3-amino-1-propaneosulfonic acid (3APS), colostrinin, scyllo-inositol and Zinc/Copper chelators have been used to prevent aggregation of Aβ. With the exception of

3APS, none of these agents have reached phase III of clinical trials. Disappointing results have since led to termination of the phase III clinical trial for 3APS[138].

Immunotherapy has been one of the most active areas of AD drug development despite an incomplete understanding of the mechanism behind amyloid clearance. Both active (i.e. vaccination) and passive immunization (i.e. monoclonal antibodies) approaches have been tested with varying levels of success. The first attempts at active immunization with Aβ1-42 yielded promising preclinical results, but led to encephalitis during clinical trials and no improvement in cognition[139]. Vaccines have since been developed to limit encephalitic adverse events, but the variable antibody response seen in older patients has prompted investigation into passive immunization. Monoclonal antibodies designed to decrease plaque formation and enhance Aβ clearance, like Bapineuzumab (Janssen/Pfizer) and Solanezumab (Eli Lilly), have been tested[140]. However, in these notable cases, they did not meet their clinical endpoints for efficacy[141,142]. It was later determined by Positron Emission Tomography (PET) that nearly one quarter of all subjects lacked amyloid pathology at baseline and that this may underlie the failure to meet efficacy outcomes[143]. Recently, results from better-designed clinical trials of the human monoclonal antibody Aducanumab (Biogen), breathed new life into the passive immunization strategy for treatment of AD. Researchers reported a dose-dependent reduction in soluble and insoluble Aβ as well as a slowing of cognitive decline[144]. The drug has now been fast-tracked to phase III clinical trials[145].

In addition to immunization strategies to promote clearance of Aβ, considerable efforts have been placed on trying to prevent production of Aβ. These strategies have focused on inhibiting β- and γ-secretase, the enzymes responsible for producing Aβ through proteolytic processing of APP. BACE1, for example, has been viewed as a prime target for therapeutics. Initial challenges in crossing the blood brain barrier have been surmounted and BACE1 inhibitors are now being developed as small molecules inhibitors with favorable pharmacokinetic profiles. Emerging evidence also indicates that these inhibitors can reduce a number of biomarkers of amyloid load. However, one notable challenge that has plagued several BACE1 inhibitors during clinical trials has been their propensity to induce off-target toxicity[146]. This is likely due to the role of BACE1 in a number of functions including axon targeting[146], myelination[147], astrogenesis/neurogenesis[148], synaptic plasticity[148], maintaining spine density[148] as well as processing of non-APP substrates like neuregulin 1[148], neural cell adhesion molecule close homolog of L1 (CHL1)[148] and the Notch-relevant Jagged 1 protein[147]. As a result, it is important to find an appropriate BACE1-inhibitor dose range where patients can balance

tolerable side-effects with the benefit of reducing Aβ sufficiently. Recently, a new class of BACE1 inhibitors have been proposed to take advantage of the subcellular distribution of BACE1-mediated APP processing. Preclinically, these inhibitors have been shown to reduce Aβ levels, but preserve BACE1 processing of non-APP substrates[100]. BACE1 inhibitors, though hindered by off-target effects, still hold promise for treatment in AD, but require further clinical validation[148].

Similar to BACE1, the γ-secretase complex has several non-APP substrates, including the Notch receptor 1. Attempts to inhibit γ-secretase have, accordingly, resulted in detrimental effects on cognition and functionality as was seen in phase III trials of Semagacestat (Eli Lilly)[149]. Notably, these trials did demonstrate a dose-dependant reduction in CSF Aβ[150]. Modulation of γ-secretase (as opposed to inhibition) has been proposed to reduce Aβ while circumventing the off-target consequence of strong inhibition.

1.6.1 Exercise

Epidemiological findings have suggested that exercise can reduce the risk of developing AD[151]. Consistently, a randomized controlled trial demonstrated that exercise can improve cognitive functioning in at-risk elderly individuals[152]. This exercise-induced improvement is likely a consequence of its actions on synapses. By affecting the number, function and structure of synapses, exercise can improve learning and memory which is prominently disrupted in AD[153]. Indeed, a recent clinical trial demonstrated the value aerobic exercise among early AD patients in improving functionality, memory and reducing hippocampal atrophy[154].

The molecular basis for these structural changes is not fully understood, but rodent-based experiments have provided several clues. Synaptic short- and long-term potentiation, for example, is increased in rats following voluntary running exercise[155]. Neurogenesis - which can result in newly formed synapses - is also enhanced after voluntary exercise[156]. In the hippocampus of adult rodents, exercise arrests neural stem cell division and drives differentiation into mature neurons. Electrophysiological measurements of these newly formed neurons indicates that the new synapses can integrate into functional circuits in the brain[157]. Moreover, exercise can protect neurons against excitotoxic and metabolic stress in experimental models of relevance to AD[158–160]. In transgenic mouse models of AD, exercise has been shown to ameliorate brain Aβ pathology and associated cognitive deficits[161–164]. The

underlying mechanisms for these benefits are unknown, but is a subject of interest in **Paper 3** of this thesis.

Activation of neurons in response to exercise results in glutamate release leading to Ca^{2+} influx via post-synaptic NMDA receptor channels. While this Ca^{2+} influx promotes oxidative stress by increasing mitochondrial superoxide production, it also activates kinase pathways (e.g. CaMK2, MAPK) in order to mediate adaptive responses including strengthening of synapses and cellular stress resistance. Additionally, the stimulated pathways activate a number of transcription factors (e.g. CREB, NF-κB, AP-1) which are linked to neuronal plasticity and survival[165]. Among the number of genes which are upregulated in response to exercise is brain-derived neurotrophic factor (BDNF)[166].

1.6.2 BDNF

BDNF is widely expressed in the CNS by both neurons and glia. The precursor form, pro-BDNF, is capable of binding to the low affinity p75 neurotrophin receptor (p75NTR) and promoting neuronal apoptosis[148]. Conversely, proteolytic processing by plasmin can convert pro-BDNF to mature BDNF, which - in turn - binds to the TrkB receptor and, thereby, promotes synaptogenesis and synaptic plasticity. As such, it plays a key function in learning and memory in the hippocampus. In AD, BDNF protein and mRNA levels are decreased[167–176] and at least one BDNF gene polymorphism (i.e. Val66Met mutation) has been shown to have a positive association with AD pathogenesis[177]. Its value as a disease-modifying strategy in neurodegenerative disorders has, accordingly, been proposed[177] and treatment with BDNF may protect against amyloid load[178,179]. However, the relationship between Aβ and BDNF remains unclear.

One line of evidence suggests that the neuroprotective feature of BDNF can protect neurons against Aβ-induced neurotoxicity[180]. For example, BDNF can restore neuronal survival and growth after pre-treatment with Aβ. In mice, this has even led to improvements in spatial learning and memory[181]. The ability of BDNF to modulate synapses may directly underlie this improvement. BDNF can increase dendritic spine formation, enhance synaptic transmission[181] and is critically involved in induction and maintenance of LTP[182–184]. Indeed, treatment with exogenous BDNF can reverse LTP deficiencies caused by Aβ[185]. This may be due to BDNFs ability to stimulate autophosphorylation of NMDA and AMPA receptors and activate CamK2 to protect synaptic function[185]. On the other hand, BDNF may alter the proteolytic processing

of APP[179] though much is still unknown regarding this process. **Paper 3** in this thesis aims to expand our understanding of this process.

2 AIMS

The overarching aim of the present thesis is to uncover mechanisms that regulate processing and trafficking of APP and APP-derived peptides. Presented below are the specific aims of constituent papers:

2.1 PAPER 1: ENDOGENOUS APP ACCUMULATES IN SYNAPSES AFTER BACE1 INHIBITION

To examine the fate of APP at neuronal synapses and the consequence of perturbing BACE1 activity.

2.2 PAPER 2: OVEREXPRESSION OF SNX3 DECREASES AMYLOID-B PEPTIDE PRODUCTION BY REDUCING ENDOCYTOSIS OF AMYLOID PRECURSOR PROTEIN

To examine the role of SNX3 in Aβ production and APP processing and trafficking.

2.3 PAPER 3: EXERCISE AND BDNF REDUCE AB PRODUCTION BY ENHANCING A-SECRETASE PROCESSING OF APP

To uncover underlying mechanisms for how exercise mediates anti-amyloidogenic functions.

2.4 PAPER 4: EXTRACELLULAR VESICLE-ASSOCIATED AB MEDIATES TRANS-NEURONAL BIOENERGETIC AND CA2+-HANDLING DEFICITS IN ALZHEIMER'S DISEASE MODELS

To elucidate the contribution of extracellular vesicles to Aβ production and pathogenesis of AD.

3 RESULTS AND DISCUSSION

3.1 PAPER 1: ENDOGENOUS APP ACCUMULATES IN SYNAPSES AFTER BACE1 INHIBITION

Synapse loss is a key pathological event in Alzheimer's Disease that correlates strongly with cognitive deficits[69]. It is thought that proteolytic processing of APP plays a central role in both pre- and postsynaptic derangements[37,186]. Notably, synapses are a major site for Aβ release[70], but β-cleavage of APP - unrelated to Aβ formation has also been implicated in synaptic impairment[187–189]. For this reason, APP processing in the context of synapses has been a subject of great interest. While trafficking and processing of APP has been studied extensively in non-neuronal cells, its precise fate in neurons and neuronal synapses has been less clear. Notably, it has been difficult to determine the exact correlation between the behavior of expressed tagged APP constructs and endogenous APP in neurons and this has led to several competing hypotheses on the trafficking of APP to synapses[99,190–192]. In **Paper 1**, we address this problem by examining endogenous APP at hippocampal synapses. This was performed by combining a variety of immunocytochemical techniques with perturbation of β-secretase.

Using antibodies directed to N-terminal APP epitopes (N-t1 and N-t2) as well as C-terminal APP epitopes (C-t1 and C-t2), immunocytochemical characterization was performed on primary neurons from mouse hippocampi. In neurons from BACE1(+/+) mice, APP N-terminus labeling showed prominent overlap with synaptic markers (synaptotagmin and SV2). APP C-terminus labeling, on the other hand, was largely separated from synapses indicating that the synaptic APP pool consists of cleaved fragments. The effects of reduced BACE1 activity were then examined using BACE(-/-) mice. The co-localization between N-terminus labeling and synaptic markers remained similar, but the peri-synaptic labeling of the C-terminus was ameliorated (Figure 4). As such, C-terminus labeling colocalized with synaptic markers and this was confirmed through two distinct quantification methods. A thresholding quantification revealed that the percentage of C-terminus labeled synapses increased 1.84 times

using the C-t1 antibody and 1.49 times using the C-t2 antibody in neurons from BACE1(-/-) mice as compared to BACE(+/+) mice. A second quantification method involved comparing the intensity of APP C-terminus labeling in a region of interest fitting the synapse to that of peri-synaptic regions. The resulting synaptic intensity ratios for both C-t1 and C-t2 were significantly higher in BACE1(-/-) neurons compared to BACE(+/+) neurons. This result suggests that the APP C-terminus labeling is specifically enriched at the synapse in BACE1(-/-) neurons. These observations represent one of two possibilities in how APP is distributed at synapses: either N-terminal and C-terminal APP fragments co-exist at synapses or there is an increase in full-length APP levels at the synapses of BACE1-/- neurons. To provide insight into which one of these two possibilities was true, biochemical measurements were performed and indicated that the latter was likely true.

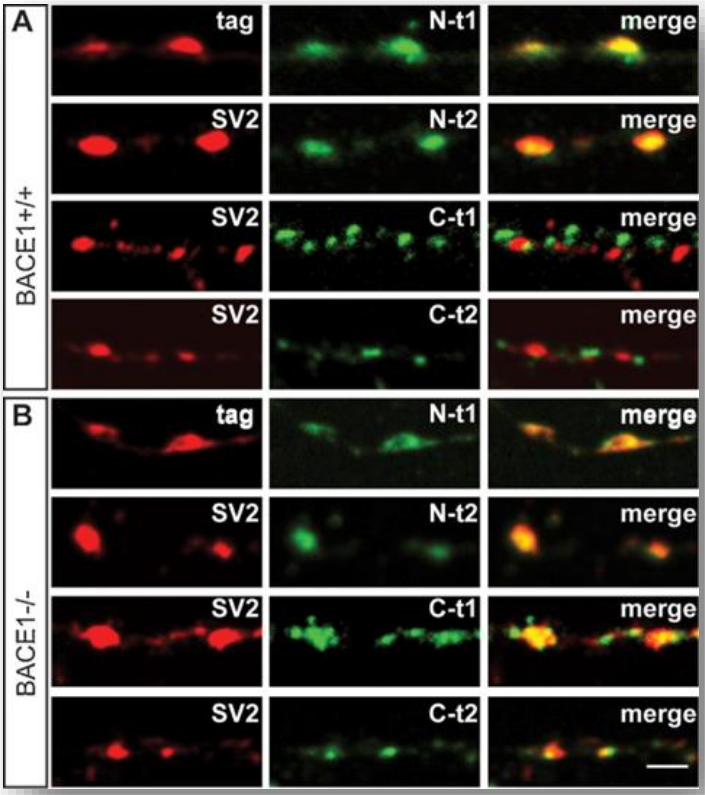

Figure 4. BACE1 deficiency induces co-accumulation of N-terminal and C-terminal APP epitopes in synaptic regions in mouse primary hippocampal neurons. (A) Double staining with synaptotagmin (tag) or SV2 antibodies and antibodies against the N-terminus of APP (N-t1, N-t2, upper panels) and C-terminus of APP (C-t1, C-t2, lower panels) in BACE1+/+ neurons. (B) Double staining with synaptotagmin or SV2 antibodies and antibodies against the N-terminus of APP (N-t1, N-t-2, upper panels) and C-terminus of APP (C-t1, C-t2, lower panels) in BACE1−/− neurons. Scale bar = 2 μm.[212]

Reducing Aβ production via pharmacological inhibition of BACE1 has been proposed as a therapeutic strategy in treatment of AD. As such, we repeated the immunocytochemical measurements in primary neurons isolated from rat hippocampi in the presence and absence of BACE1 inhibitor. In control neurons, APP N-terminus labeling was tightly co-localized with synaptic markers whereas APP C-terminus labeling was adjacent to synaptic regions. BACE1 inhibitor treatment did not affect the distribution of APP N-terminus labeling, but did enhance colocalization of the APP C-terminus with synaptic markers. Thresholding quantification revealed a significantly higher percentage of synapses labeled with C-t1 and C-t2 compared to

control neurons. Additionally, synaptic intensity ratios were significantly higher in BACE1 inhibitor treated neurons as compared to control. Cumulatively, these findings are consistent with results obtained from BACE1-/- mice.

To assess whether this C-terminus labeling shift can occur at the presynapse, we turned to proximity ligation assay (PLA) as the resolution of this technique allows distinction between the pre- and postsynaptic compartments. Antibodies directed towards APP C-terminus were ligated with antibodies directed towards presynaptic marker SV2. The resulting ligation was amplified producing a fluorescent signal. The number of PLA signals per synaptic region were then quantified - revealing a significant increase in PLA signals following BACE1 inhibition. Accordingly, this observation indicates that the APP C-terminus shift can occur presynaptically.

Our findings, involving the study of endogenous APP in neurons, suggest that full-length APP can be trafficked through axons and targeted to synaptic vesicles of the presynapse. This is consistent with studies using APP constructs that have suggested that the enzymatic machinery needed for production of Aβ is operative in the presynaptic compartment[193]. Because the synapse is viewed as a critical site for Aβ release and synaptic loss is a key pathological event in AD, our findings provide support for targeting components of the presynaptic endosomal pathway in treatment of AD. An important implication of our observations is that part of the synaptic defects seen with potent BACE1 inhibitors[194,195] may be due to accumulation of full-length APP at the synapse.

3.2 PAPER 2: OVEREXPRESSION OF SNX3 DECREASES AMYLOID-B PEPTIDE PRODUCTION BY REDUCING ENDOCYTOSIS OF AMYLOID PRECURSOR PROTEIN

Sorting nexins (SNXs) have diverse functions in protein sorting and membrane trafficking. A single nucleotide polymorphism in SNX3 was recently found to be associated with AD[196]. However it remains unknown how SNX3 participates in Aβ production. In **Paper 2**, we explore this open question by studying the behavior of APP in response to SNX3 overexpression in human embryonic kidney cell line 293T (HEK293T).

Using the highly sensitive and specific MesoScale Discovery multiplex ELISA system, we found that SNX3 overexpression together with APP in HEK293T cells significantly reduced the levels of both secreted Aβ40 and Aβ42. Moreover, the SNX3 overexpression also decreased

sAPPβ levels in cell culture media. These findings cumulatively suggest that SNX3 overexpression reduces amyloidogenic processing of APP.

To understand the mechanism for this reduction, we sought to understand the relationship between APP and BACE1 in intact cells. To this end, a bimolecular fluorescence complementation (BiFC) assay was used. APP was tagged with the non-fluorescent N-terminal fragment of the Venus protein (VN), while BACE1 is tagged with the non-fluorescent C-terminal fragment of the Venus protein (VC). When APP and BACE1 are in close proximity, VN and VC come together to form a functional Venus protein that produces a fluorescent signal. Our results indicate that overexpression of SNX3 caused a significant reduction of the interaction between APP and BACE1.

Because many lines of evidence suggest that endosomes are the critical site for BACE1-mediated processing of APP, we tested whether SNX3 overexpression led to an inhibition of APP endocytosis. To this effect, APP was N-terminally tagged with an α-bungarotoxin-binding site (BBS). Endocytosis of APP was investigated by incubating cell surface BBS-APP with Alexa Fluor 555-conjugated α-bungarotoxin. Compared to control cells, overexpression of SNX3 significantly reduced APP internalization (Figure 5). Accordingly, it would be expected that SNX3 overexpression would lead to enhanced APP levels at the cell surface. To test this possibility, cell surface APP levels were determined by flow cytometry. Indeed, SNX3 overexpression led to an increase in the percentage of cells labeled with surface APP. These results, cumulatively, suggest that SNX3 regulates Aβ production by mediating internalization of APP from the surface to intracellular processing organelles.

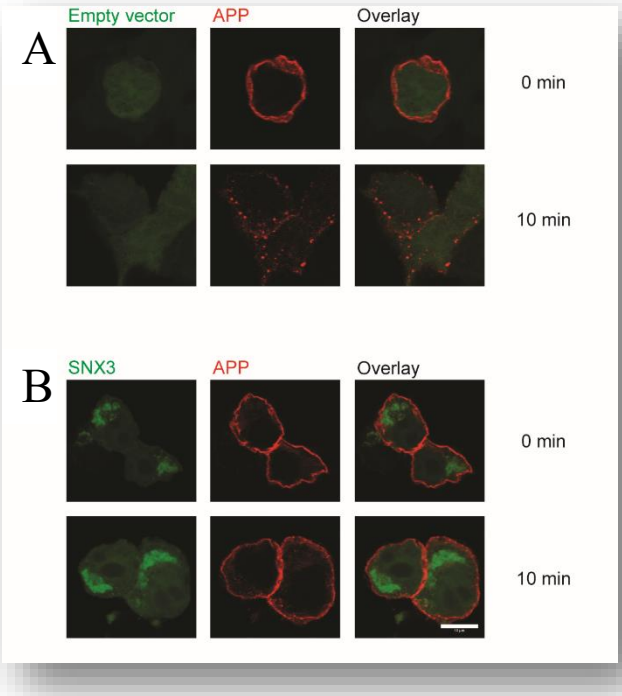

Figure 5. Overexpression of SNX3 inhibits endocytosis of APP. HEK293T cells were co-transfected with empty vector (A) or SNX3 (B) and BBS-APP, and endocytosis of cell surface APP was examined. Scale bar = 10 μm.

Our findings are consistent with previous studies suggesting that SNX3

is involved in endocytosis of cargo molecules[197,198]. Although the precise role of SNX3 in endocytosis is unclear, our findings provide evidence that it can incluence APP endocytosis and, as such, may be a putative target in AD treatment research. Moreover, previous studies have also demonstrated that a close homolog of SNX3, SNX12, regulates Aβ production by affecting endocytosis of BACE1[98]. Thus, there may be distinct sorting pathways for APP vs. APP-relevant secretases. Targeting SNXs would, therefore, be particularly appealing if it reduces amyloidogenic processing of APP whilst leaving BACE1 processing of non-APP substrates intact.

3.3 PAPER 3: EXERCISE AND BDNF REDUCE AB PRODUCTION BY ENHANCING A-SECRETASE PROCESSING OF APP

Epidemiological findings suggest that regular exercise can reduce the risk of AD, and studies in AD mouse models have demonstrated that running wheel exercise can reduce Aβ accumulation and ameliorate cognitive deficits[165,199]. Despite these findings, the underlying cellular and molecular mechanisms are unknown. A large body of evidence suggests that brain-derived neurotrophic factor (BDNF) regulates key adaptive responses with regards to exercise. These responses include neurogenesis, synapse formation, learning and memory, and neuronal stress resistance - many of which are perturbed in the pathological AD state[200,201]. Indeed, AD patients have lower levels of mature BDNF compared to age-matched control subjects[173]. Cumulatively, these findings highlight the therapeutic potential of BDNF in treatment of AD despite the fact that it is not clear exactly how BDNF affects APP processing. **Paper 3** of this thesis aims to address these open questions by examining the relationship between exercise, BDNF and APP processing in AD mice as well as neural cells.

To test whether a relatively short 3-week period of exercise is sufficient to reduce Aβ levels in AD mice, a voluntary running wheel experiment was performed. Following 3 weeks, hippocampi from runner mice and sedentary control mice were isolated. Aβ40 and Aβ42 levels were then measured in these samples using the highly sensitive and specific MesoScale Discovery multiplex ELISA system. We found significant reduction in both Aβ isoforms in exercising mice compared to sedentary controls. Moreover, we found that sAPPα levels were also increased in exercising mice. Cumulatively, these observations indicated that exercise shifts the processing of APP to favor the non-amyloidogenic pathway. Because BDNF is considered a prominent mediator of exercise-induced effects, including in counteracting neurodegenerative processes in animal models of AD, we turned our attention to BDNF.

Indeed, in our exercising mice, BDNF levels were elevated approximately 2-fold in comparison to sedentary control mice (Figure 6).

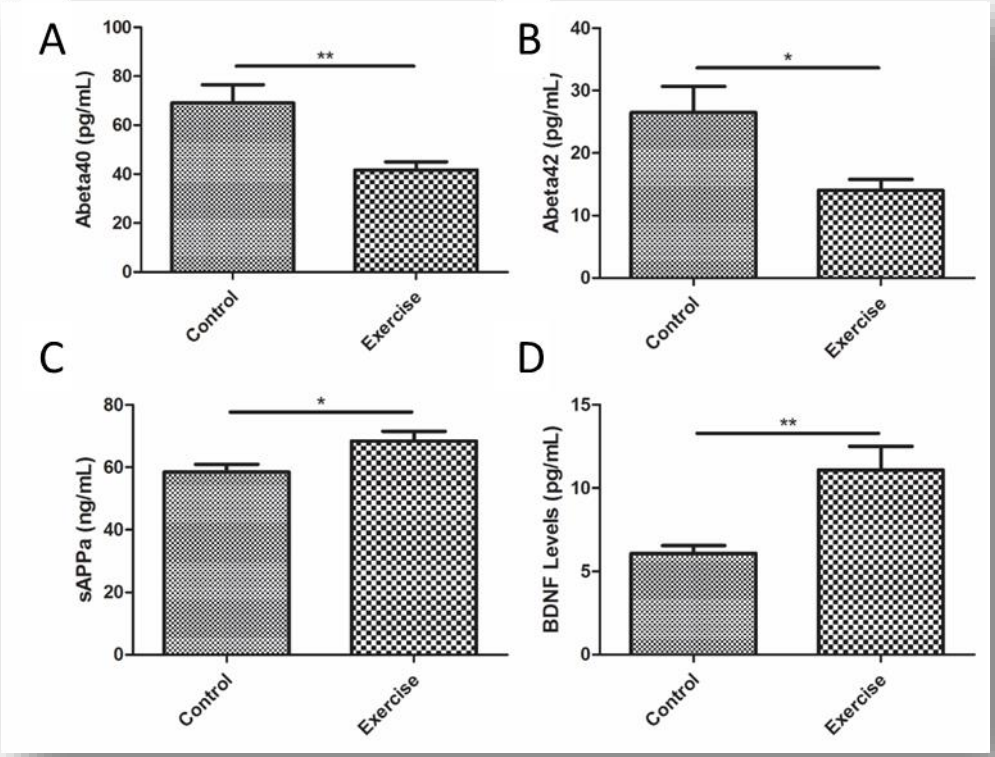

Figure 6. Exercise shifts APP processing towards the non-amyloidogenic pathway. 3 weeks of voluntary running wheel exercise among AD mice significantly lowered hippocampal Aβ40 (A) and Aβ42 (B) levels while significantly raising sAPPα (C) and BDNF (D) levels compared to sedentary control mice.[213]

To test whether BDNF itself can recapture the Aβ observations seen with exercise, we treated human SHSY5Y neural cells with BDNF. As was seen with exercise, levels of secreted Aβ40 and Aβ42 in culture media were significantly reduced compared to control. Therefore, it appears that BDNF is able to suppress amyloidogenic load - though the mechanism is unclear. We hypothesized that BDNF reduces Aβ through modulation of the α-secretase enzyme. To test this hypothesis, an α-secretase inhibitor was used alone and in combination with BDNF treatment. Treatment with α-secretase inhibitor significantly increased secreted Aβ levels compared to control cultures. Importantly, in cultures co-treated with α-secretase inhibitor and BDNF, Aβ levels were significantly higher than control cultures. Moreover, BDNF treatment enhanced sAPPα levels. Taken together, these results indicate that BDNF likely reduces Aβ levels by enhancing α-secretase activity.

The subcellular localization of α-secretase plays a role in its proteolytic ability to process APP. Because the cell surface is thought of as the primary site for α-secretase processing of APP, we

hypothesized that BDNF increases the amount of α-secretase on the cell surface. Using flow cytometry, we found that BDNF treatment actually resulted in reduced cell-surface α-secretase. This surprising finding supports the notion that regulated α-secretase activity occurs intracellularly while constitutive α-secretase activity occurs at the cell surface[202]. Indeed cellular levels of sAPPα were enhanced in BDNF treated cultures compared to control.

Although sAPPα has been linked to neurite outgrowth[19], it is not clear whether BDNF utilizes the peptide to carry out its neurotrophic functions. To test the hypothesis that BDNF promotes neurite outgrowth through enhanced sAPPα production, SHSY5Y neural cells were cultured in a manner to produce a homogenous population of neuronally differentiated cells. A fluorescence based neurite outgrowth assay was used in conjunction with BDNF and α-secretase inhibitor treatment. BDNF resulted in significant enhancement of neurite outgrowth compared to control cultures. However, BDNF and α-secretase inhibitor co-treatment did not significantly alter neurite outgrowth as compared to cultures treated with BDNF alone. This finding indicates that there are likely multiple mechanisms by which BDNF promotes neurite outgrowth and enhancement of α-secretase is likely only a minor contributor.

BDNF has been proposed as a therapeutic in AD primarily due to its ability to promote neuronal survival and neurite outgrowth. However, our work also suggests its value as a prophylactic. By regulating α-secretase activity, BDNF plays an important role in determining the fate of APP with regards to APP processing pathways. Importantly, sAPPα has recently been shown to bind to and inhibit BACE1[203]. This finding suggests the presence of an endogenous autoregulatory positive feedback loop to promote non-amyloidogenic processing of APP and our study provides a scenario for this to happen. Taking advantage of such a loop, for example via BDNF, might offer a compelling prophylactic strategy in treatment of AD as it would likely limit off-target adverse events traditionally seen with BACE1 inhibitors. Because BDNF may have low blood-barrier penetration and poor biostability[204], it is important to identify factors that influence BDNF production in vivo. Exercise, which enhances BDNF in brain cells, is therefore an attractive approach for increasing α-secretase activity in the fight against AD.

3.4 PAPER 4: EXTRACELLULAR VESICLE-ASSOCIATED AB MEDIATES TRANS-NEURONAL BIOENERGETIC AND CA2+-HANDLING DEFICITS IN ALZHEIMER'S DISEASE MODELS

It has recently been suggested that pathogenic forms of Aβ can propagate between cells in a prion-like manner. Thus, the misfolded protein is thought to pass from donor to recipient cell

where it can function as a seed for further aggregation[205,206]. The mechanisms for this propagation is, however, unclear[207]. Extracellular vesicles (EVs), including exosomes, represent one potential mechanism by which pathogenic protein can be spread throughout the brain. Multivesicular bodies, being a part of the endosomal system, have been reported as an important site for Aβ generation and, for this reason, EVs may also be a site of APP processing into Aβ[58,208]. **Paper 4** of the present thesis aims to expand our understanding of the contribution of EVs to Aβ production and AD pathogenesis.

We first isolated EVs from the cell media of H4 glioblastoma cell lines expressing either a pathogenic presenilin mutation or wildtype presenilin according to established guidelines[48]. Aβ levels were then measured using the MesoScale Discovery multiplex ELISA system. We found that while both Aβ40 and Aβ42 were significantly lower in the EVs compared to the EV-depleted media, the Aβ42/Aβ40 ratio in EVs was significantly higher than in EV-depleted media. This holds particular relevance as the Aβ42/Aβ40 ratio is considered a better predictor of AD than individual concentrations of Aβ isoforms[209,210]. Similar Aβ results were obtained when isolating exosomes released by human neurons derived from induced pluripotent stems cells of an AD patient carrying a pathogenic presenilin mutation compared to cells from a normal human subject. By treating EVs with trypsin, we tested whether Aβ is associated to the outer surface membrane of EVs or contained within EVs. A reduction in both Aβ40 and Aβ42 following trypsin treatment suggests that Aβ binds to the surface of EVs. Notably, our results indicates that this association with the EV surface likely occurs prior to EV release from cells.

Because Aβ production is intimately linked to the endosomal system, we hypothesized that inhibiting the lysosome would increase the release of Aβ42-containing EVs from multivesicular bodies to the extracellular space. Indeed, incubating H4 cells with an inhibitor of lysosomal function (bafilomycin A) increased EV secretion as well as the EV Aβ42/Aβ40 ratio. This holds particular relevance as lysosomal dysfunction has previously been shown to occur in AD patients[211].

To examine whether these EVs derived from cells containing pathogenic presenilin mutation are neurotoxic, EVs were isolated from H4 culture media and added to the media of primary rat cortical neurons. Significant reduction in neuronal viability occurred within 48 hours and the cells were more sensitive to glutamate-induced excitotoxicity. Moreover, treatment of primary neurons with EVs derived from H4 cells with pathogenic presenilin mutations resulted

in perturbed Ca^{2+} handling capacity as well mitochondrial impairment. Collectively, these events may lead to the observed neurotoxicity.

To determine whether EVs isolated from AD patients and animal models exhibit the same neurotoxic properties as EVs isolated from cell cultures, the levels of EV Aβ40 and Aβ42 were determined from the plasma of AD mice and CSF of AD patients. In both cases, the levels of each individual Aβ isoform in EVs was lower than in EV-depleted plasma/CSF. However, as was the case with results from cell culture, there was a significantly higher Aβ42/Aβ40 ratio in EVs compared to the EV-depleted control fractions. CSF-derived EVs from AD patients were then incubated with cultured cortical neurons and underwent a battery of tests to examine neurotoxicity. Significant reduction in neuronal viability, increase in vulnerability to glutamate-induced excitotoxicity, enhanced Aβ aggregation and apoptosis, perturbed Ca^{2+} handling capacity and reduced mitochondrial respiration were observed.

Cumulatively, these findings highlight the potential role of EVs in propagating Aβ-related pathology and associated neuronal degeneration. Notably, EV-mediated neurotoxicity is likely the consequence of heightened Aβ42/Aβ40 ratio at the surface of EVs rather than the concentration individual Aβ isoforms. In view of the fact that lysosome dysfunction is increased in AD patients, EVs may represent a compensatory mechanisms for handling amyloid load. Moreover, amyloid overload may promote cells to use EVs as a mechanism for disposing intracellular Aβ and, accordingly, lessen the burden on lysosomes. While this may lessen the amyloid burden on the cell, neighboring cells may be exposed to EV-induced neurotoxicity. As such, targeting Aβ-laden EVs may hold potential as an AD therapeutic.

4 CONCLUSIONS AND FUTURE PERSPECTIVES

This thesis provides insight into mechanisms which regulate amyloid precursor protein processing and trafficking. **Paper 1** determines that endogenous full-length APP is - in fact - capable of trafficking to presynaptic nerve terminals and accumulates upon BACE1 perturbation. This observation highlights the importance of monitoring synaptic response when developing BACE1 inhibitors to prevent off-target toxicity. **Paper 2** identifies a novel protein, SNX3, which is capable of transporting APP and, thereby, regulates amyloidogenic processing. Further validation - both in primary neuronal cells and in AD animal models - may provide insight into whether this could serve as a suitable target in AD therapy. **Paper 3** concludes that exercise and BDNF can reduce toxic Aβ production by enhancing α-secretase processing of APP. This enhancement appears to be the consequence of α-secretase distribution and may offer a scenario for an endogenous autoregulatory feedback loop to commence and, accordingly, suppress amyloidogenesis. Exercise and BDNF may, therefore, be capable of "jump-starting" the cellular proteolysis system in a fashion to correct pathogenic mechanisms in AD. **Paper 4** concludes that extracellular vesicles in AD relevant models carry a higher ratio of Aβ42/Aβ40 than their surroundings and can induce neurotoxicity of neighboring cells. In light of this, extracellular vesicles are currently being explored for their diagnostic utility as well as a method for understanding how amyloidogenic propagation can occur within the brain.

While all 4 papers hold value in the development of AD therapeutics, Paper 3 is one that can have near-immediate implications and would be my personal choice for furtherment. Although it is apparent that both exercise and BDNF have beneficial effects in reducing Aβ through enhancement of the non-amyloidogenic pathway for APP processing, it would be interesting to identify whether the key regulator for exercise-induced Aβ effects is, in fact, BDNF. To this effect, in vivo running experiments involving AD mice with perturbed BDNF signaling could offer additional support. Moreover, sedentary AD mice treated with BDNF/BDNF mimetics/trkB agonist could open the door for an exciting class of novel biopharmaceutical drugs.

Clinical trials have demonstrated the value of exercise as a strategy in fighting AD-related impairments and the start-up costs of such strategies are considerably lower than drug development. As such, I believe promoting exercise among at-risk individuals to be an exciting approach in alleviating AD symptoms. Though exercise is traditionally well-

regarded in its ability to fight - for example - weight gain and heart disease, its benefit in memory and cognition is becoming increasingly difficult to ignore. Education regarding the neurobiological benefits of exercise should, therefore, be a salient goal for both scientists and healthcare providers worldwide.

The UK-based National Health Service estimates that people aged over 65 years, on average, spend 10 or more hours a day sitting/lying down making them the most sedentary age group. As such, they describe inactivity as a "silent killer" for the generation. Because physical limitations among this age group may hinder participation in traditional forms of exercise, an interesting avenue of further research involves comparison between different exercise modalities in their propensity to help in AD. For example, while running may place a heightened demand on bones and joints, swimming could serve as a more suitable, low-impact alternative. Collaboration between physicians, physical therapists and basic research scientists could help in design of tailored regimens that are effective both prophylactically and therapeutically in the context of AD.

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6 REFERENCES

1. Alzheimer's Association. *2017 Alzheimer's Disease Facts and Figures*. (2017). at <http://www.alz.org/facts/>

2. Hippius, H. & Neundörfer, G. The discovery of Alzheimer's disease. *Dialogues Clin. Neurosci.* **5,** 101–108 (2003).

3. Selkoe, D. J. Toward a comprehensive theory for Alzheimer's disease. Hypothesis: Alzheimer's disease is caused by the cerebral accumulation and cytotoxicity of amyloid beta-protein. *Ann. N. Y. Acad. Sci.* **924,** 17–25 (2000).

4. Hardy, J. A. & Higgins, G. A. Alzheimer's disease: The Amyloid Cascade Hypothesis. 184–185 doi:10.1126/science.1566067

5. Alzheimer's Association. Alzheimer's & Brain Research Milestones. at <http://www.alz.org/research/science/major_milestones_in_alzheimers.asp>

6. Teplow, D. B. Structural and kinetic features of amyloid beta-protein fibrillogenesis. *Amyloid Int. J. Exp. Clin. Investig. Off. J. Int. Soc. Amyloidosis* **5,** 121–142 (1998).

7. Iwatsubo, T. *et al.* Visualization of Aβ42(43) and Aβ40 in senile plaques with end-specific Aβ monoclonals: evidence that an initially deposited species is Aβ42(43). *Neuron* **13,** 45–53 (1994).

8. Selkoe, D. J. & Hardy, J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol. Med.* **8,** 1–14 (2016).

9. Kang, J. *et al.* The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* **325,** 733–736 (1987).

10. Jacobsen, K. T. & Iverfeldt, K. Amyloid precursor protein and its homologues: A family of proteolysis-dependent receptors. *Cellular and Molecular Life Sciences* **66,** 2299–2318 (2009).

11. Mattson, M. P. Cellular actions of β-amyloid precursor protein and its soluble and fibrillogenic derivatives. *Physiol. Rev.* **77,** 1081–1132 (1997).

12. Zheng, H. *et al.* β-amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. *Cell* **81,** 525–531 (1995).

13. Heber, S. *et al.* Mice with Combined Gene Knock-outs Reveal Essential and Partially Redundant Functions of Amyloid Precursor Protein Family Members. *J. Neurosci.* **20,** 7951–7963 (2000).

14. von Koch, C. S. *et al.* Generation of APLP2 KO mice and early postnatal lethality in APLP2/APP double KO mice. *Neurobiol. Aging* **18,** 661–669 (1997).

15. Eggert, S. *et al.* The proteolytic processing of the amyloid precursor protein gene family members APLP-1 and APLP-2 involves α-, β-, γ-, and ε-Like cleavages: Modulation of APLP-1 processing by N-glycosylation. *J. Biol. Chem.* **279,** 18146–18156 (2004).

16. O'Brien, R. J. & Wong, P. C. Amyloid precursor protein processing and Alzheimer's disease. *Annu. Rev. Neurosci.* **34,** 185–204 (2011).

17. Willem, M. *et al.* η-Secretase processing of APP inhibits neuronal activity in the hippocampus. *Nature* **526,** 443–447 (2015).

18. Mattson, M. P. *et al.* Evidence for Excitoprotective and Intraneuronal Calcium-Regulating Roles for Secreted Forms of the β-Amyloid Precursor Protein. *Neuron* **I,** 243–254 (1993).

19. Gakhar-Koppole, N. *et al.* Activity requires soluble amyloid precursor protein α to promote neurite outgrowth in neural stem cell-derived neurons via activation of the MAPK pathway. *Eur. J. Neurosci.* **28,** 871–882 (2008).

20. Ishida, A., Furukawa, K., Keller, J. N. & Mattson, M. P. Secreted form of β-amyloid precursor protein shifts the frequency dependency for induction of LTD, and enhances LTP in hippocampal slices. *Neuroreport* **8,** 2133–7 (1997).

21. Meziane, H. *et al.* Memory-enhancing effects of secreted forms of the β-amyloid precursor protein in normal and amnestic mice. *Proc. Natl. Acad. Sci. U. S. A.* **95,** 12683–12688 (1998).

22. Taylor, C. J. *et al.* Endogenous secreted amyloid precursor protein-α regulates hippocampal NMDA receptor function, long-term potentiation and spatial memory. *Neurobiol. Dis.* **31,** 250–260 (2008).

23. Obregon, D. *et al.* Soluble amyloid precursor protein-α modulates β-secretase activity and amyloid-β generation. *Nat. Commun.* **3,** 777 (2012).

24. Almkvist, O. *et al.* Cerebrospinal fluid levels of α-secretase-cleaved soluble amyloid precursor protein mirror cognition in a Swedish family with Alzheimer disease and a gene mutation. *Arch. Neurol.* **54,** 641–644 (1997).

25. Brinkmalm, G. *et al.* Soluble amyloid precursor protein α and β in CSF in Alzheimer's disease. *Brain Res.* **1513,** 117–26 (2013).

26. Habib, A., Sawmiller, D. & Tan, J. Restoring Soluble Amyloid Precursor Protein α Functions as a Potential Treatment for Alzheimer's Disease. *J. Neurosci. Res.* **991,** 973–991 (2016).

27. Kuhn, P.-H. *et al.* ADAM10 is the physiologically relevant, constitutive α-secretase of the amyloid precursor protein in primary neurons. *EMBO J.* **29,** 3020–3032 (2010).

28. Postina, R. Activation of α-secretase cleavage. *J. Neurochem.* **120 Suppl,** 46–54 (2012).

29. Chow, V. W., Mattson, M. P., Wong, P. C. & Gleichmann, M. An overview of APP processing enzymes and products. *Neuromolecular Med.* **12,** 1–12 (2010).

30. Słomnicki, Ł. P. & Leśniak, W. A putative role of the Amyloid Precursor Protein Intracellular Domain (AICD) in transcription. *Acta Neurobiol. Exp. (Wars).* **68,** 219–228 (2008).

31. Furukawa, K., Barger, S. & Blalock, E. Activation of K+ channels and suppression of neuronal activity by secreted β-amyloid-precursor protein. (1996). doi:8538744

32. Nikolaev, A., McLaughlin, T., O'Leary, D. D. M. & Tessier-Lavigne, M. APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. *Nature* **457,** 981–9 (2009).

33. Li, H. *et al.* Soluble amyloid precursor protein (APP) regulates transthyretin and Klotho gene expression without rescuing the essential function of APP. *Proc. Natl. Acad. Sci. U. S. A.* **107,** 17362–7 (2010).

34. Weyer, S. W. *et al.* APP and APLP2 are Essential at PNS and CNS Synapses for Transmission, Spatial Learning and LTP. *EMBO J.* **30,** 2266–2280 (2011).

35. Chasseigneaux, S. & Allinquant, B. Functions of Aβ, sAPPα and sAPPβ: Similarities and differences. *J. Neurochem.* **120,** 99–108 (2012).

36. Shaw, L. M. *et al.* Cerebrospinal fluid biomarker signature in alzheimer's disease neuroimaging initiative subjects. *Ann. Neurol.* **65,** 403–413 (2009).

37. Haass, C., Kaether, C., Thinakaran, G. & Sisodia, S. Trafficking and Proteolytic Processing of APP. *Cold Spring Harb. Perspect. Med.* **2,** a006270 (2012).

38. Sisodia, S. S. β-amyloid precursor protein cleavage by a membrane-bound protease. *Proc. Natl. Acad. Sci. U. S. A.* **89,** 6075–9 (1992).

39. Lai, A., Sisodia, S. S. & Trowbridge, I. S. Characterization of sorting signals in the beta-amyloid precursor protein cytoplasmic domain. *J. Biol. Chem.* **270,** 3565–3573 (1995).

40. Cirrito, J. R. *et al.* Endocytosis is required for synaptic activity-dependent release of amyloid-β in vivo. *Neuron* **58,** 42–51 (2008).

41. Jiang, S. *et al.* Trafficking regulation of proteins in Alzheimer ' s disease. *Mol. Neurodegener.* **9,** 6 (2014).

42. Xiao, T. *et al.* The role of exosomes in the pathogenesis of Alzheimer' disease. *Transl. Neurodegener.* **6,** 3 (2017).

43. Kosaka, N. *et al.* Secretory mechanisms and intercellular transfer of microRNAs in living cells. *J. Biol. Chem.* **285,** 17442–17452 (2010).

44. Sharples, R. A. *et al.* Inhibition of γ-secretase causes increased secretion of amyloid precursor protein C-terminal fragments in association with exosomes. *FASEB J.* **22,** 1469–1478 (2008).

45. Gupta, A. & Pulliam, L. Exosomes as mediators of neuroinflammation. *J. Neuroinflammation* **11,** 68 (2014).

46. Eitan, E., Suire, C., Zhang, S. & Mattson, M. P. Impact of lysosome status on extracellular vesicle content and release. *Ageing Res. Rev.* **32,** 65–74 (2016).

47. Colombo, M., Raposo, G. & Théry, C. Biogenesis, Secretion, and Intercellular Interactions of Exosomes and Other Extracellular Vesicles. *Annu. Rev. Cell Dev. Biol* **30,** 255–89 (2014).

48. Lötvall, J. *et al.* Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for

Extracellular Vesicles. *J. Extracell. vesicles* **3,** 26913 (2014).

49. Colombo, M. *et al.* Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. *J. Cell Sci.* **126,** 5553–5565 (2013).

50. Stenmark, H. Rab GTPases as coordinators of vesicle traffic. *Nat. Rev. Mol. Cell Biol.* **10,** 513–525 (2009).

51. Hsu, C. *et al.* Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A-C. *J. Cell Biol.* **189,** 223–232 (2010).

52. Tytell, M., Lasek, R. J. & Gainer, H. Axonal maintenance, glia, exosomes, and heat shock proteins. *F1000Research* **5,** 205 (2016).

53. Lamichhane, T. N., Raiker, R. S. & Jay, S. M. Exogenous DNA Loading into Extracellular Vesicles via Electroporation is Size-Dependent and Enables Limited Gene Delivery. *Mol. Pharm.* **12,** 3650–3657 (2015).

54. Huang, X. *et al.* Characterization of human plasma-derived exosomal RNAs by deep sequencing. *BMC Genomics* **14,** 319 (2013).

55. Jenjaroenpun, P. *et al.* Characterization of RNA in exosomes secreted by human breast cancer cell lines using next-generation sequencing. *PeerJ* **1,** e201 (2013).

56. Miao, Y., Li, G., Zhang, X., Xu, H. & Abraham, S. N. A TRP Channel Senses Lysosome Neutralization by Pathogens to Trigger Their Expulsion. *Cell* **161,** 1306–1319 (2015).

57. Baixauli, F., Lopez-Otin, C. & Mittelbrunn, M. Exosomes and autophagy: Coordinated mechanisms for the maintenance of cellular fitness. *Front. Immunol.* **5,** 1–6 (2014).

58. Rajendran, L. *et al.* Alzheimer's disease β-amyloid peptides are released in association with exosomes. *Proc. Natl. Acad. Sci. U. S. A.* **103,** 11172–7 (2006).

59. Perez-Gonzalez, R., Gauthier, S. A., Kumar, A. & Levy, E. The exosome secretory pathway transports amyloid precursor protein carboxyl-terminal fragments from the cell into the brain extracellular space. *J. Biol. Chem.* **287,** 43108–43115 (2012).

60. Saman, S. *et al.* Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. *J. Biol.*

Chem. **287,** 3842–3849 (2012).

61. Nixon, R. A. The role of autophagy in neurodegenerative disease. *Nat. Med.* **19,** 983–997 (2013).

62. Yuyama, K., Sun, H., Mitsutake, S. & Igarashi, Y. Sphingolipid-modulated exosome secretion promotes clearance of amyloid-β by microglia. *J. Biol. Chem.* **287,** 10977–10989 (2012).

63. Vingtdeux, V., Sergeant, N. & Buee, L. Potential contribution of exosomes to the prion-like propagation of lesions in Alzheimer's disease. *Front. Physiol.* **3,** 229 (2012).

64. Dinkins, M. B., Dasgupta, S., Wang, G., Zhu, G. & Bieberich, E. Exosome reduction in vivo is associated with lower amyloid plaque load in the 5XFAD mouse model of Alzheimer's disease. *Neurobiol. Aging* **35,** 1792–1800 (2014).

65. Bellingham, S. A., Guo, B. B., Coleman, B. M. & Hill, A. F. Exosomes: vehicles for the transfer of toxic proteins associated with neurodegenerative diseases? *Front. Physiol.* **3,** 124 (2012).

66. An, K. *et al.* Exosomes neutralize synaptic-plasticity-disrupting activity of Aβ assemblies in vivo. *Mol. Brain* **6,** 47 (2013).

67. Yuyama, K. *et al.* Decreased amyloid-β pathologies by intracerebral loading of glycosphingolipid-enriched exosomes in Alzheimer model mice. *J. Biol. Chem.* **289,** 24488–24498 (2014).

68. Yuyama, K. *et al.* A potential function for neuronal exosomes: Sequestering intracerebral amyloid-β peptide. *FEBS Lett.* **589,** 84–88 (2015).

69. Serrano-Pozo, A., Frosch, M. P., Masliah, E. & Hyman, B. T. Neuropathological alterations in Alzheimer disease. *Cold Spring Harb. Perspect. Med.* **1,** a006189 (2011).

70. Cirrito, J. R. *et al.* Synaptic activity regulates interstitial fluid amyloid-β levels in vivo. *Neuron* **48,** 913–22 (2005).

71. Lazarov, O. *et al.* Axonal transport, amyloid precursor protein, kinesin-1, and the processing apparatus: revisited. *J. Neurosci.* **25,** 2386–2395 (2005).

72. Shankar, G. M. *et al.* Amyloid-β protein dimers isolated directly from Alzheimer's

brains impair synaptic plasticity and memory. *Nat. Med.* **14,** 837–842 (2008).

73. Frykman, S. *et al.* Synaptic and endosomal localization of active γ-secretase in rat brain. *PLoS One* **5,** e8948 (2010).

74. Kandalepas, P. C. *et al.* The Alzheimer's β-secretase BACE1 localizes to normal presynaptic terminals and to dystrophic presynaptic terminals surrounding amyloid plaques. *Acta Neuropathol.* **126,** 329–52 (2013).

75. Teasdale, R. D., Loci, D., Houghton, F., Karlsson, L. & Gleeson, P. A. A large family of endosome-localized proteins related to sorting nexin 1. *Biochem. J.* **358,** 7–16 (2001).

76. Carlton, J., Bujny, M., Rutherford, A. & Cullen, P. Sorting nexins--unifying trends and new perspectives. *Traffic* **6,** 75–82 (2005).

77. Schöbel, S. *et al.* A novel sorting nexin modulates endocytic trafficking and α-secretase cleavage of the amyloid precursor protein. *J. Biol. Chem.* **283,** 14257–14268 (2008).

78. Wang, X. *et al.* Sorting Nexin 27 Regulates Aβ Production through Modulating γ-Secretase Activity. *Cell Rep.* **9,** 1023–1033 (2014).

79. Cullen, P. J. Endosomal sorting and signalling: an emerging role for sorting nexins. *Nat. Rev. Mol. Cell Biol.* **9,** 574–82 (2008).

80. Parvathy, S., Hussain, I., Karran, E. H., Turner, A. J. & Hooper, N. M. Cleavage of Alzheimer ' s Amyloid Precursor Protein by R -Secretase Occurs at the Surface of Neuronal Cells †. 9728–9734 (1999).

81. Skovronsky, D. M., Moore, D. B., Milla, M. E., Doms, R. W. & Lee, V. M. Protein Kinase C-dependent α -Secretase Competes with β-Secretase for Cleavage of Amyloid- β Precursor Protein in the Trans-Golgi Network. *J. Biol. Chem.* **275,** 2568–2575 (2000).

82. Lichtenthaler, S. F. Alpha-secretase in Alzheimer's disease: Molecular identity, regulation and therapeutic potential. *J. Neurochem.* **116,** 10–21 (2011).

83. Kojro, E. *et al.* The neuropeptide PACAP promotes the α-secretase pathway for processing the Alzheimer amyloid precursor protein. *FASEB J.* **20,** 512–4 (2006).

84. Bandyopadhyay, S., Goldstein, L. E., Lahiri, D. K. & Rogers, J. T. Role of the APP non-amyloidogenic signaling pathway and targeting α-secretase as an alternative drug target for treatment of Alzheimer's disease. *Curr. Med. Chem.* **14,** 2848–2864 (2007).

85. Marcello, E., Gardoni, F., Di Luca, M. & Pérez-Otan, I. An arginine stretch limits ADAM10 exit from the endoplasmic reticulum. *J. Biol. Chem.* **285,** 10376–10384 (2010).

86. Vassar, R. BACE1: the β-secretase enzyme in Alzheimer's disease. *J. Mol. Neurosci.* **23,** 105–114 (2004).

87. Yang, L.-B. *et al.* Elevated β-secretase expression and enzymatic activity detected in sporadic Alzheimer disease. *Nature medicine* **9,** 3–4 (2003).

88. Sinha, S. *et al.* Purification and cloning of amyloid precursor protein β-secretase from human brain. *Nature* **402,** 537–540 (1999).

89. Vassar, R. *et al.* β-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* **286,** 735–741 (1999).

90. Cai, H. *et al.* BACE1 is the major β-secretase for generation of Aβ peptides by neurons. *Nat. Neurosci.* **4,** 233–234 (2001).

91. Lau, K. F., McLoughlin, D. M., Standen, C. & Miller, C. C. X11α and X11β interact with presenilin-1 via their PDZ domains. *Mol. Cell. Neurosci.* **16,** 557–565 (2000).

92. Shi, X. P. *et al.* The pro domain of β-secretase does not confer strict zymogen-like properties but does assist proper folding of the protease domain. *J. Biol. Chem.* **276,** 10366–10373 (2001).

93. Huse, J. T., Pijak, D. S., Leslie, G. J., Lee, V. M. & Doms, R. W. Maturation and endosomal targeting of β-site amyloid precursor protein-cleaving enzyme. The Alzheimer's disease β-secretase. *J. Biol. Chem.* **275,** 33729–33737 (2000).

94. Hook, V. Y. H. *et al.* β-amyloid peptide in regulated secretory vesicles of chromaffin cells: evidence for multiple cysteine proteolytic activities in distinct pathways for β-secretase activity in chromaffin vesicles. *J. Neurochem.* **81,** 237–256 (2002).

95. von Arnim, C. A. F. *et al.* Demonstration of BACE (beta-secretase) phosphorylation and its interaction with GGA1 in cells by fluorescence-lifetime imaging microscopy. *J. Cell Sci.* **117,** 5437–5445 (2004).

96. Wahle, T. *et al.* GGA proteins regulate retrograde transport of BACE1 from endosomes to the trans-Golgi network. *Mol. Cell. Neurosci.* **29,** 453–461 (2005).

97. Okada, H. *et al.* Proteomic identification of sorting nexin 6 as a negative regulator of BACE1-mediated APP processing. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **24,** 2783–2794 (2010).

98. Zhao, Y. *et al.* Sorting nexin 12 interacts with BACE1 and regulates BACE1-mediated APP processing. *Mol. Neurodegener.* **7,** 30 (2012).

99. Sannerud, R. *et al.* ADP ribosylation factor 6 (ARF6) controls amyloid precursor protein (APP) processing by mediating the endosomal sorting of BACE1. *Proc. Natl. Acad. Sci. U. S. A.* **108,** E559-68 (2011).

100. Ben Halima, S. *et al.* Specific Inhibition of β-Secretase Processing of the Alzheimer Disease Amyloid Precursor Protein. *Cell Rep.* **14,** 2127–2141 (2016).

101. Kimberly, W. T. *et al.* γ-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2. *Proc. Natl. Acad. Sci. U. S. A.* **100,** 6382–6387 (2003).

102. St George-Hyslop, P. & Fraser, P. E. Assembly of the presenilin γ-/ε-secretase complex. *J. Neurochem.* **120 Suppl,** 84–88 (2012).

103. Dries, D. R. & Yu, G. Assembly, maturation, and trafficking of the γ-secretase complex in Alzheimer's disease. *Curr. Alzheimer Res.* **5,** 132–46 (2008).

104. Prasher, V. P. *et al.* Molecular mapping of Alzheimer-type dementia in Down's syndrome. *Ann. Neurol.* **43,** 380–383 (1998).

105. Bateman, R. J. *et al.* Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N. Engl. J. Med.* **367,** 795–804 (2012).

106. Jonsson, T. *et al.* A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature* **488,** 96–9 (2012).

107. Alonso Vilatela, M. E., López-López, M. & Yescas-Gómez, P. Genetics of Alzheimer's disease. *Arch. Med. Res.* **43,** 622–31 (2012).

108. Lemere, C. A. *et al.* The E280A presenilin 1 Alzheimer mutation produces increased A beta 42 deposition and severe cerebellar pathology. *Nat. Med.* **2,** 1146–1150 (1996).

109. Corder, E. H. *et al.* Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261,** 921–923 (1993).

110. Roses, A. D. Apolipoprotein E and Alzheimer's disease. A rapidly expanding field with medical and epidemiological consequences. *Ann. N. Y. Acad. Sci.* **802,** 50–57 (1996).

111. Liu, C.-C., Kanekiyo, T., Xu, H. & Bu, G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat. Rev. Neurol.* **9,** 106–118 (2013).

112. Kosik, K. S., Joachim, C. L. & Selkoe, D. J. Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. *Proc. Natl. Acad. Sci. U. S. A.* **83,** 4044–4048 (1986).

113. Maccioni, R. B., Vera, J. C., Dominguez, J. & Avila, J. A discrete repeated sequence defines a tubulin binding domain on microtubule-associated protein tau. *Arch. Biochem. Biophys.* **275,** 568–579 (1989).

114. Ghoshal, N. *et al.* Tau conformational changes correspond to impairments of episodic memory in mild cognitive impairment and Alzheimer's disease. *Exp. Neurol.* **177,** 475–493 (2002).

115. Maccioni, R. B. *et al.* Anomalously phosphorylated tau and Aβ fragments in the CSF correlates with cognitive impairment in MCI subjects. *Neurobiol. Aging* **27,** 237–244 (2006).

116. Gonzalez, C., Farias, G. & Maccioni, R. B. Modification of tau to an Alzheimer's type protein interferes with its interaction with microtubules. *Cell. Mol. Biol. (Noisy-le-grand).* **44,** 1117–1127 (1998).

117. Farías, G., González-Billault, C. & Maccioni, R. B. Immunological characterization of epitopes on tau of Alzheimer's type and chemically modified tau. *Mol. Cell. Biochem.* **168,** 59–66 (1997).

118. Tampellini, D. & Gouras, G. K. Synapses, synaptic activity and intraneuronal Aβ in Alzheimer's disease. *Front. Aging Neurosci.* **2,** 1–5 (2010).

119. Berger, Z. *et al.* Accumulation of pathological tau species and memory loss in a conditional model of tauopathy. *J. Neurosci.* **27,** 3650–3662 (2007).

120. Maeda, S. *et al.* Granular tau oligomers as intermediates of tau filaments. *Biochemistry*

46, 3856–3861 (2007).

121. Maccioni, R. B., Farías, G., Morales, I. & Navarrete, L. The Revitalized Tau Hypothesis on Alzheimer's Disease. *Arch. Med. Res.* **41,** 226–231 (2010).

122. Edrey, Y. H. & Salmon, A. B. Revisiting an age-old question regarding oxidative stress. *Free Radic. Biol. Med.* **71,** 368–378 (2014).

123. Perry, G., Cash, A. D. & Smith, M. a. Alzheimer Disease and Oxidative Stress. *J. Biomed. Biotechnol.* **2,** 120–123 (2002).

124. Premkumar, D. R. *et al.* Induction of heme oxygenase-1 mRNA and protein in neocortex and cerebral vessels in Alzheimer's disease. *J. Neurochem.* **65,** 1399–1402 (1995).

125. Smith, M. A. *et al.* Heme oxygenase-1 is associated with the neurofibrillary pathology of Alzheimer's disease. *Am. J. Pathol.* **145,** 42–47 (1994).

126. Markesbery, W. R. Oxidative stress hypothesis in Alzheimer's disease. *Free Radic. Biol. Med.* **23,** 134–147 (1997).

127. Gregersen, N. & Bross, P. Protein misfolding and cellular stress: an overview. *Methods Mol. Biol.* **648,** 3–23 (2010).

128. Zotova, E., Nicoll, J. A. R., Kalaria, R., Holmes, C. & Boche, D. Inflammation in Alzheimer ' s disease: relevance to pathogenesis and therapy. *Alzheimer's Res. Ther.* 1–9 (2010).

129. Hayes, A. *et al.* A polymorphic variation in the interleukin 1A gene increases brain microglial cell activity in Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* **75,** 1475–1477 (2004).

130. Rich, J. B. *et al.* Nonsteroidal anti-inflammatory drugs in Alzheimer's disease. *Neurology* **45,** 51–55 (1995).

131. De La Torre, J. C. The vascular hypothesis of Alzheimer's disease: Bench to bedside and beyond. *Neurodegener. Dis.* **7,** 116–121 (2010).

132. Wood, W. G., Li, L., Müller, W. E. & Eckert, G. P. Cholesterol as a causative factor in Alzheimer's disease: A debatable hypothesis. *J. Neurochem.* **129,** 559–572 (2014).

133. Bush, A. I. & Tanzi, R. E. Therapeutics for Alzheimer's disease based on the metal

hypothesis. *Neurotherapeutics* **5,** 421–432 (2008).

134. Neve, R. L. & McPhie, D. L. The cell cycle as a therapeutic target for Alzheimer's disease. *Pharmacol. Ther.* **111,** 99–113 (2006).

135. Parsons, C. G., Danysz, W., Dekundy, A. & Pulte, I. Memantine and cholinesterase inhibitors: Complementary mechanisms in the treatment of Alzheimer's disease. *Neurotox. Res.* **24,** 358–369 (2013).

136. Parsons, C. G., Danysz, W. & Quack, G. Memantine is a clinically well tolerated N-methyl-D-aspartate (NMDA) receptor antagonist - a review of preclinical data. *Neuropharmacology* **38,** 735–767 (1999).

137. Berk, C. & Sabbagh, M. N. Successes and failures for drugs in late-stage development for Alzheimer's disease. *Drugs Aging* **30,** 783–92 (2013).

138. Yiannopoulou, K. G. & Papageorgiou, S. G. Current and future treatments for Alzheimer's disease. *Ther Adv Neurol Disord* **6,** 19–33 (2013).

139. Gilman, S. *et al.* Clinical effects of Aβ immunization (AN1792) in patients with AD in an interrupted trial. *Neurology* **64,** 1553–1562 (2005).

140. Moreth, J., Mavoungou, C. & Schindowski, K. Passive anti-amyloid immunotherapy in Alzheimer's disease: What are the most promising targets? *Immun. Ageing* **10,** 18 (2013).

141. Pfizer Pharmaceutical. Pfizer Announces Co-Primary Clinical Endpoints Not Met In Second Phase 3 Bapineuzumab Study In Mild-To-Moderate Alzheimer's Disease Patients Who Do Not Carry The Apoe4 Genotype. 5–6 (2012).

142. Eli Lilly. Lilly Announces Top-Line Results of Solanezumab Phase 3 Clinical Trial. 2015–2017 (2017).

143. Alzforum. Phase 3 Trial Data for Solanezumab and Bapineuzumab Released. at <http://www.alzforum.org/news/research-news/phase-3-trial-data-solanezumab-and-bapineuzumab-released>

144. Sevigny, J. *et al.* The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. *Nature* **537,** 50–56 (2016).

145. 221AD301 Phase 3 Study of Aducanumab (BIIB037) in Early Alzheimer's Disease. at

<https://clinicaltrials.gov/ct2/show/NCT02477800?term=BIIB037&rank=3>

146. Vassar, R. BACE1 inhibitor drugs in clinical trials for Alzheimer's disease. *Alzheimers. Res. Ther.* **6,** 89 (2014).

147. Hu, X., He, W., Luo, X., Tsubota, K. E. & Yan, R. BACE1 Regulates Hippocampal Astrogenesis via the Jagged1-Notch Pathway. *Cell Rep.* **4,** 40–49 (2013).

148. Hu, X., He, W., Luo, X., Tsubota, K. E. & Yan, R. BACE1 Regulates Hippocampal Astrogenesis via the Jagged1-Notch Pathway. *Cell Rep.* **4,** 40–49 (2013).

149. Imbimbo, B. P. & Giardina, G. A. M. γ-secretase inhibitors and modulators for the treatment of Alzheimer's disease: disappointments and hopes. *Curr. Top. Med. Chem.* **11,** 1555–1570 (2011).

150. Siemers, E. *et al.* Safety, tolerability, and changes in amyloid β concentrations after administration of a γ-secretase inhibitor in volunteers. *Clin. Neuropharmacol.* **28,** 126–132 (2005).

151. Norton, S., Matthews, F. E., Barnes, D. E., Yaffe, K. & Brayne, C. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. *Lancet. Neurol.* **13,** 788–794 (2014).

152. Ngandu, T. *et al.* A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): A randomised controlled trial. *Lancet* **385,** 2255–2263 (2015).

153. Kim, S. J. & Linden, D. J. Ubiquitous plasticity and memory storage. *Neuron* **56,** 582–592 (2007).

154. Morris, J. K. *et al.* Aerobic exercise for Alzheimer's disease: A randomized controlled pilot trial. *PLoS One* **12,** e0170547 (2017).

155. Farmer, J. *et al.* Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male Sprague-Dawley rats in vivo. *Neuroscience* **124,** 71–79 (2004).

156. van Praag, H., Kempermann, G. & Gage, F. H. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat. Neurosci.* **2,** 266–270 (1999).

157. van Praag, H. *et al.* Functional neurogenesis in the adult hippocampus. *Nature* **415,** 1030–1034 (2002).

158. Reiss, J. I., Dishman, R. K., Boyd, H. E., Robinson, J. K. & Holmes, P. V. Chronic activity wheel running reduces the severity of kainic acid-induced seizures in the rat: possible role of galanin. *Brain Res.* **1266,** 54–63 (2009).

159. Lau, Y.-S., Patki, G., Das-Panja, K., Le, W.-D. & Ahmad, S. O. Neuroprotective effects and mechanisms of exercise in a chronic mouse model of Parkinson's disease with moderate neurodegeneration. *Eur. J. Neurosci.* **33,** 1264–1274 (2011).

160. Cheng, A. *et al.* Mitochondrial SIRT3 Mediates Adaptive Responses of Neurons to Exercise and Metabolic and Excitatory Challenges. *Cell Metab.* **23,** 128–142 (2016).

161. Adlard, P. A., Perreau, V. M., Pop, V. & Cotman, C. W. Voluntary Exercise Decreases Amyloid Load in a Transgenic Model of Alzheimer ' s Disease. **25,** 4217–4221 (2005).

162. Um, H. S. *et al.* Exercise training acts as a therapeutic strategy for reduction of the pathogenic phenotypes for Alzheimer's disease in an NSE/APPsw-transgenic model. *Int. J. Mol. Med.* **22,** 529–539 (2008).

163. Moore, K. M. *et al.* A spectrum of exercise training reduces soluble Aβ in a dose-dependent manner in a mouse model of Alzheimer's disease. *Neurobiol. Dis.* **85,** 218–224 (2016).

164. Ozbeyli, D. *et al.* Protective effects of different exercise modalities in an Alzheimer's disease-like model. *Behav. Brain Res.* **328,** 159–177 (2017).

165. Mattson, M. P. Energy intake and exercise as determinants of brain health and vulnerability to injury and disease. *Cell Metab.* **16,** 706–722 (2012).

166. Stranahan, A. M. *et al.* Voluntary exercise and caloric restriction enhance hippocampal dendritic spine density and BDNF levels in diabetic mice. *Hippocampus* **19,** 951–961 (2009).

167. Ventriglia, M. *et al.* Serum brain-derived neurotrophic factor levels in different neurological diseases. *Biomed Res. Int.* **2013,** 901082 (2013).

168. Nagata, T. *et al.* Plasma BDNF levels are correlated with aggressiveness in patients with amnestic mild cognitive impairment or Alzheimer disease. *J. Neural Transm.*

121, 433–441 (2014).

169. Boiocchi, C. *et al.* Brain-derived neurotrophic factor gene variants and Alzheimer disease: an association study in an Alzheimer disease Italian population. *Rejuvenation Res.* **16,** 57–66 (2013).

170. Phillips, H. S. *et al.* BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. *Neuron* **7,** 695–702 (1991).

171. Connor, B. *et al.* Brain-derived neurotrophic factor is reduced in Alzheimer's disease. *Brain Res. Mol. Brain Res.* **49,** 71–81 (1997).

172. Ferrer, I. *et al.* BDNF and full-length and truncated TrkB expression in Alzheimer disease. Implications in therapeutic strategies. *J. Neuropathol. Exp. Neurol.* **58,** 729–739 (1999).

173. Hock, C., Heese, K., Hulette, C., Rosenberg, C. & Otten, U. Region-specific neurotrophin imbalances in Alzheimer disease: decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. *Arch Neurol* **57,** (2000).

174. Holsinger, R. M., Schnarr, J., Henry, P., Castelo, V. T. & Fahnestock, M. Quantitation of BDNF mRNA in human parietal cortex by competitive reverse transcription-polymerase chain reaction: decreased levels in Alzheimer's disease. *Brain Res. Mol. Brain Res.* **76,** 347–354 (2000).

175. Garzon, D., Yu, G. & Fahnestock, M. A new brain-derived neurotrophic factor transcript and decrease in brain-derived neurotrophic factor transcripts 1, 2 and 3 in Alzheimer's disease parietal cortex. *J. Neurochem.* **82,** 1058–1064 (2002).

176. Peng, S., Wuu, J., Mufson, E. J. & Fahnestock, M. Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. *J. Neurochem.* **93,** 1412–1421 (2005).

177. Voineskos, A. N. *et al.* The brain-derived neurotrophic factor Val66Met polymorphism and prediction of neural risk for Alzheimer disease. *Arch. Gen. Psychiatry* **68,** 198–206 (2011).

178. Nagahara, A. H. *et al.* Early BDNF treatment ameliorates cell loss in the entorhinal cortex of APP transgenic mice. *J. Neurosci.* **33,** 15596–15602 (2013).

179. Rohe, M. *et al.* Brain-derived neurotrophic factor reduces amyloidogenic processing through control of SORLA gene expression. *J. Neurosci.* **29,** 15472–8 (2009).

180. Nagahara, A. H. *et al.* Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. *Nat. Med.* **15,** 331–337 (2009).

181. Zhou, J. P. *et al.* Transduced PTD-BDNF fusion protein protects against β amyloid peptide-induced learning and memory deficits in mice. *Brain Res.* **1191,** 12–19 (2008).

182. Lu, B., Nagappan, G. & Lu, Y. BDNF and synaptic plasticity, cognitive function, and dysfunction. *Handb. Exp. Pharmacol.* **220,** 223–250 (2014).

183. Lee, P. R., Cohen, J. E., Becker, K. G. & Fields, R. D. Gene expression in the conversion of early-phase to late-phase long-term potentiation. *Ann. N. Y. Acad. Sci.* **1048,** 259–271 (2005).

184. Monteggia, L. M. *et al.* Essential role of brain-derived neurotrophic factor in adult hippocampal function. *Proc. Natl. Acad. Sci. U. S. A.* **101,** 10827–10832 (2004).

185. Zeng, Y., Zhao, D. & Xie, C.-W. Neurotrophins enhance CaMKII activity and rescue amyloid-β-induced deficits in hippocampal synaptic plasticity. *J. Alzheimers. Dis.* **21,** 823–831 (2010).

186. Selkoe, D., Mandelkow, E. & Holtzman, D. Deciphering Alzheimer disease. *Cold Spring Harb. Perspect. Med.* **2,** a011460 (2012).

187. Gouras, G. K., Tampellini, D., Takahashi, R. H. & Capetillo-Zarate, E. Intraneuronal β-amyloid accumulation and synapse pathology in Alzheimer's disease. *Acta Neuropathol.* **119,** 523–41 (2010).

188. Mucke, L. & Selkoe, D. J. Neurotoxicity of Amyloid β-Protein: Synaptic and Network Dysfunction. *Cold Spring Harb. Perspect. Med.* **2,** a006338 (2012).

189. Tamayev, R., Matsuda, S., Arancio, O. & D'Adamio, L. β- but not γ-secretase proteolysis of APP causes synaptic and memory deficits in a mouse model of dementia. *EMBO Mol. Med.* **4,** 171–9 (2012).

190. Cirrito, J. R. *et al.* Endocytosis is required for synaptic activity-dependent release of amyloid-β in vivo. *Neuron* **58,** 42–51 (2008).

191. Groemer, T. W. *et al.* Amyloid precursor protein is trafficked and secreted via synaptic

vesicles. *PLoS One* **6,** e18754 (2011).

192. Marquez-Sterling, N. R., Lo, a C., Sisodia, S. S. & Koo, E. H. Trafficking of cell-surface β-amyloid precursor protein: evidence that a sorting intermediate participates in synaptic vesicle recycling. *J. Neurosci.* **17,** 140–51 (1997).

193. DeBoer, S. R., Dolios, G., Wang, R. & Sisodia, S. S. Differential Release of -Amyloid from Dendrite- Versus Axon-Targeted APP. *J. Neurosci.* **34,** 12313–12327 (2014).

194. Laird, F. M. *et al.* BACE1, a major determinant of selective vulnerability of the brain to amyloid-β amyloidogenesis, is essential for cognitive, emotional, and synaptic functions. *J. Neurosci.* **25,** 11693–11709 (2005).

195. Wang, H., Song, L., Laird, F., Wong, P. C. & Lee, H.-K. BACE1 knock-outs display deficits in activity-dependent potentiation of synaptic transmission at mossy fiber to CA3 synapses in the hippocampus. *J. Neurosci.* **28,** 8677–81 (2008).

196. Vardarajan, B. N. *et al.* Identification of Alzheimer disease-associated variants in genes that regulate retromer function. *Neurobiol. Aging* **33,** 2231.e15-2231.e30 (2012).

197. Boulkroun, S. *et al.* Vasopressin-inducible ubiquitin-specific protease 10 increases ENaC cell surface expression by deubiquitylating and stabilizing sorting nexin 3. *Am. J. Physiol. Renal Physiol.* **295,** F889-900 (2008).

198. Strochlic, T. I., Setty, T. G., Sitaram, A. & Burd, C. G. Grd19/Snx3p functions as a cargo-specific adapter for retromer-dependent endocytic recycling. *J. Cell Biol.* **177,** 115–125 (2007).

199. Adlard, P. A., Perreau, V. M., Pop, V. & Cotman, C. W. Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. *J. Neurosci.* **25,** 4217–4221 (2005).

200. Intlekofer, K. A. & Cotman, C. W. Exercise counteracts declining hippocampal function in aging and Alzheimer's disease. *Neurobiol. Dis.* **57,** 47–55 (2013).

201. Marosi, K. & Mattson, M. P. BDNF mediates adaptive brain and body responses to energetic challenges. *Trends Endocrinol. Metab.* **25,** 89–98 (2014).

202. Skovronsky, D. M., Moore, D. B., Milla, M. E., Doms, R. W. & Lee, V. M. Protein Kinase C-dependent α -Secretase Competes with β-Secretase for Cleavage of Amyloid- β Precursor Protein in the Trans-Golgi Network. **275,** 2568–2575 (2000).

203. Obregon, D. *et al.* Soluble amyloid precursor protein-α modulates β-secretase activity and amyloid-β generation. *Nat. Commun.* **3,** 777 (2012).

204. Song, J.-H., Yu, J.-T. & Tan, L. Brain-Derived Neurotrophic Factor in Alzheimer's Disease: Risk, Mechanisms, and Therapy. *Mol. Neurobiol.* **52,** 1477–1493 (2015).

205. Aguzzi, A. & Rajendran, L. The transcellular spread of cytosolic amyloids, prions, and prionoids. *Neuron* **64,** 783–790 (2009).

206. Watts, J. C. *et al.* Serial propagation of distinct strains of Aβ prions from Alzheimer's disease patients. *Proc. Natl. Acad. Sci. U. S. A.* **111,** 10323–10328 (2014).

207. Stohr, J. *et al.* Purified and synthetic Alzheimer's amyloid β (Aβ) prions. *Proc. Natl. Acad. Sci. U. S. A.* **109,** 11025–11030 (2012).

208. Takahashi, R. H. *et al.* Intraneuronal Alzheimer Aβ42 accumulates in multivesicular bodies and is associated with synaptic pathology. *Am. J. Pathol.* **161,** 1869–1879 (2002).

209. Spies, P. E. *et al.* The cerebrospinal fluid amyloid β42/40 ratio in the differentiation of Alzheimer's disease from non-Alzheimer's dementia. *Curr. Alzheimer Res.* **7,** 470–476 (2010).

210. Sauvee, M. *et al.* Additional use of Aβ42/Aβ40 ratio with cerebrospinal fluid biomarkers P-tau and Aβ42 increases the level of evidence of Alzheimer's disease pathophysiological process in routine practice. *J. Alzheimers. Dis.* **41,** 377–386 (2014).

211. Wolfe, D. M. *et al.* Autophagy failure in Alzheimer's disease and the role of defective lysosomal acidification. *Eur. J. Neurosci.* **37,** 1949–1961 (2013).

212. Nigam, S. M. *et al.* Endogenous APP accumulates in synapses after BACE1 inhibition. *Neurosci. Res.* **109,** 9–15 (2016).

213. Nigam, S. M. *et al.* Exercise and BDNF reduce Aβ production by enhancing α-secretase processing of APP. *J. Neurochem.* **38,** 42–49 (2017).